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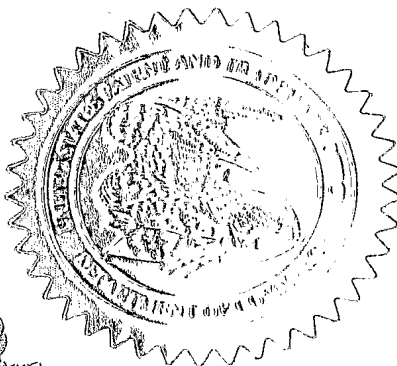
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

Express Mail Label No.

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Michael	Tesar	Weilheim, Germany

☐ Additional inventors are being named on the _____ separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

Anti-CD38 Human Antibodies and Uses Therefor

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ENCLOSED APPLICATION PARTS (check all that apply)☒ Specification Number of Pages

55

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18

☐ Other (specify)☒ Application Data Sheet. See 37 CFR 1.76**METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT**☒ Applicant claims small entity status. See 37 CFR 1.27.☒ A check or money order is enclosed to cover the filing feesFILING FEE
AMOUNT (\$)☐ The Commissioner is hereby authorized to charge filing
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08-1641

\$80

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____Respectfully submitted,
SIGNATURE

Date

2/26/04

TYPED or PRINTED NAME

Paul M. Booth

REGISTRATION NO.
(if appropriate)

40,244

Docket Number:

37629-0085

TELEPHONE

202-912-2000

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FEE TRANSMITTAL for FY 2003

Effective 01/01/2003. Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27

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METHOD OF PAYMENT (check one)

☒ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

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FEE CALCULATION

1. BASIC FILING FEE

Large Fee Code	Entity Fee (\$)	Small Fee Code	Entity Fee (\$)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80

SUBTOTAL (1) (\$ 80)

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

Total Claims	Extra Claims	Fee from below	Fee Paid
Independent Claims	-20** = 0	x 0 = 0	0
Multiple Dependent	-3** = 0	x 0 = 0	0

Large Fee Code	Entity Fee (\$)	Small Fee Code	Entity Fee (\$)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	**Reissue independent claims over original patent
1205	18	2205	9	**Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) (\$)

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FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Fee Code	Entity Fee (\$)	Small Fee Code	Entity Fee (\$)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for <i>ex parte</i> reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify)

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SUBTOTAL (3) (\$)

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Attorney Docket No. 37629-0085A

Inventor: Michael Tesar
Residence: Weilheim, German
Citizenship: German

Anti-CD38 Human Antibodies

and

Uses Therefor

BACKGROUND OF THE INVENTION

CD38 is a type-II membrane glycoprotein and belongs to the family of ectoenzymes, due to its enzymatic activity as ADP ribosyl-cyclase and cADP-hydrolase. During ontogeny, CD38 appears on CD34⁺ committed stem cells and lineage-committed progenitors of lymphoid, erythroid and myeloid cells. It is understood that CD38 expression persists only in the lymphoid lineage, through the early stages of T- and B-cell development.

The up-regulation of CD38 serves as a marker for lymphocyte activation—in particular B-cell differentiation along the plasmacytoid pathway. (Co-)receptor functions of CD38 leading to intracellular signaling or intercellular communication via its ligand, CD31, are postulated, as well as its role as an intracellular regulator of a second messenger, cyclic ADPr, in a variety of signaling cascades. However, its physiological importance remains to be elucidated, since knock out of the murine analogue or anti-CD38 auto-antibodies in humans do not appear to be detrimental.

Apart from its expression in the hematopoietic system, researchers have noted the up-regulation of CD38 on various cell-lines derived from B-, T-, and myeloid/monocytic tumors, including B- or T-cell acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), Non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM). In MM, for example, strong CD38 expression is witnessed in the majority of all patient samples.

Hence, over-expression of CD38 on malignant cells provides an attractive therapeutic target for immunotherapy. Of special attraction is the fact that the most primitive pluripotent stem cells of the hematopoietic system are CD38-negative and that the extent of cytotoxic effects by ADCC or CDC correlates well with the expression-levels of the respective target.

Current approaches of anti-CD38 therapies can be divided in two groups: in vivo and ex vivo approaches. In in vivo approaches, anti-CD38 antibodies are administered to a subject in need of therapy in order to cause the antibody-mediated depletion of CD38-overexpressing malignant cells. Depletion can either be achieved by antibody-mediated ADCC and/or CDC by effector cells, or by using the anti-CD38 antibodies as targeting moieties for the transport of cytotoxic substances, e.g. saporin, to the target cells, and subsequent internalization. In the ex vivo approach, cell population, e.g. bone marrow cells, comprising CD38 overexpressing malignant cells are removed from an individual in need of treatment and are contacted with anti-CD38 antibodies. The target cells are either destroyed by cytotoxic substances, e.g. saporin, as described for the in vivo approach, or are removed by contacting the cell population with immobilized anti-CD38 antibodies, thus removing CD38 overexpressing target cells from the mixture. Thereafter, the depleted cell population is reinserted into the patient.

Antibodies specific for CD38 can be divided in different groups, depending on various properties. Binding of some antibodies to the CD38 molecule (predominantly aa 220-300) can trigger activities within the target cell, such as Ca^{2+} release, cytokine release, phosphorylation events and growth stimulation based on the respective antibody specificity (Konopleva et al., 1998; Ausiello et al 2000), but no clear correlation between the binding site of the various known antibodies and their (non-)agonistic properties could be seen (Funaro et al., 1990). However, all antibodies recognize seem to exclusively recognize epitopes located in the C-terminal part of CD38 (amino acid residues 220 to 300. No antibodies are known so far, which would be specific for epitopes in the N-terminal part of CD38 far distant from the active site in the primary protein sequence.

Relatively little is known about the efficacy of published anti-CD38 antibodies. However, we have found that OKT10, which has been in clinical testing, has a relatively low affinity and efficacy when analyzed as chimeric construct comprising a human Fc part. Furthermore, OKT10 is a murine
5 antibody, so that any chimeric or humanized OKT10-based antibody construct would still comprise sequences of murine origin in at least the variable domains.

A human anti-CD38 scFv antibody fragment has recently been described (WO 02/06347). However, that antibody is specific for a subset of CD38 molecules only.

10 Correspondingly, in light of the great potential for anti-CD38 antibody therapy, there is a high need for human anti-CD38 antibodies with high affinity and with high efficacy in mediating killing of CD38 overexpressing malignant cells by ADCC and/or CDC.

The present invention satisfies these and other needs by providing fully
15 human and highly efficacious anti-CD38 antibodies, which are described below.

SUMMARY OF THE INVENTION

It is an object of the invention to provide human antibodies that can effectively mediate the killing of CD38-overexpressing cells.

20 It is another object of the invention to provide antibodies that are safe for human administration.

It is also an object of the present invention to provide methods for treating disease or and/or conditions associated with CD38 up-regulation by using one or more antibodies of the invention. These and other objects of the invention are
25 more fully described herein.

In one aspect, the invention provides an isolated human antibody or functional antibody fragment that contains an antigen-binding region that is specific for an epitope of CD38, where the human antibody or functional fragment thereof is able to mediate killing of a CD38+ target cell (LP-1 (DSMZ: ACC41) and RPMI-8226 (ATCC: CCL-155)) by antibody-dependent cellular cytotoxicity ("ADCC") with an at least two-to five-fold better efficacy than the chimeric OKT10 antibody having SEQ ID NOS: 23 and 24 (under the same or substantially the same conditions), when a human PBMC cell is employed as an effector cell, and when the ratio of target cells to effector cells is between about 30:1 and about 50:1. Such an antibody or functional fragment thereof may contain an antigen-binding region that contains an H-CDR3 region depicted in SEQ ID NO: 5, 6, 7, or 8; the antigen-binding region may further include an H-CDR2 region depicted in SEQ ID NO: 5, 6, 7, or 8; and the antigen-binding region also may contain an H-CDR1 region depicted in SEQ ID NO: 5, 6, 7, or 8. Such a CD38-specific antibody of the invention may contain an antigen-binding region that contains an L-CDR3 region depicted in SEQ ID NO: 13, 14, 15, or 16; the antigen-binding region may further include an L-CDR1 region depicted in SEQ ID NO: 13, 14, 15, or 16; and the antigen-binding region also may contain an L-CDR2 region depicted in SEQ ID NO: 13, 14, 15, or 16.

In another aspect, the invention provides an isolated human antibody or functional antibody fragment that contains an antigen-binding region that is specific for an epitope of CD38, where the human antibody or functional fragment thereof is able to mediate killing of a CD38-transfected CHO cell by CDC with an at least two-fold better efficacy than chimeric OKT10 (SEQ ID NOS: 23 and 24) under the same or substantially the same conditions. An antibody satisfying these criteria may contain an antigen-binding region that contains an H-CDR3 region

depicted in SEQ ID NO: 5, 6, or 7; the antigen-binding region may further include an H-CDR2 region depicted in SEQ ID NO: 5, 6, or 7; and the antigen-binding region also may contain an H-CDR1 region depicted in SEQ ID NO: 5, 6, or 7. Such a CD38-specific antibody of the invention may contain an antigen-binding
5 region that contains an L-CDR3 region depicted in SEQ ID NO: 13, 14, or 15; the antigen-binding region may further include an L-CDR1 region depicted in SEQ ID NO: 13, 14, or 15; and the antigen-binding region also may contain an L-CDR2 region depicted in SEQ ID NO: 13, 14, or 15.

Antibodies (and functional fragments thereof) of the invention may
10 contain an an antigen-binding region that is specific for an epitope of CD38, which epitope contains one or more amino acid residues of amino acid residues 1 to 215 of CD38 as depicted by SEQ ID NO: 22. More specifically, an epitope to which the antigen-binding region binds may contain one or more amino acid residues found in one or more of the amino acid stretches taken from the list of
15 amino acid stretches 44-66, 82-94, 142-154, 148-164, 158-170, and 192-206. For certain antibodies, the epitope may be linear, whereas for others, it may be conformational (*i.e.*, discontinuous). An antibody or functional fragment thereof having one or more of these properties may contain an antigen-binding region that contains an H-CDR3 region depicted in SEQ ID NO: 5, 6, 7, or 8; the antigen-
20 binding region may further include an H-CDR2 region depicted in SEQ ID NO: 5, 6, 7, or 8; and the antigen-binding region also may contain an H-CDR1 region depicted in SEQ ID NO: 5, 6, 7, or 8. Such a CD38-specific antibody of the invention may contain an antigen-binding region that contains an L-CDR3 region depicted in SEQ ID NO: 13, 14, 15, or 16; the antigen-binding region may further
25 include an L-CDR1 region depicted in SEQ ID NO: 13, 14, 15, or 16; and the

antigen-binding region also may contain an L-CDR2 region depicted in SEQ ID NO: 13, 14, 15, or 16.

Peptide variants of the sequences disclosed herein are also embraced by the present invention. Accordingly, the invention includes human anti-CD38
5 antibodies having a heavy chain amino acid sequence with: at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 6, 7, or 8; and/or at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 6, 7, or 8. Further included are human anti-CD38 antibodies having a light chain amino acid
10 sequence with: at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 14, 15 or 16; and/or at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 14, 15 or 16.

An antibody of the invention may be an IgG (*e.g.*, IgG₁), while an
15 antibody fragment may be a Fab or scFv, for example. An inventive antibody fragment, accordingly, may be, or may contain, an antigen-binding region that behaves in one or more ways as described herein.

The invention also is related to isolated nucleic acid sequences, each of which can encode an antigen-binding region of a human antibody or functional
20 fragment thereof that is specific for an epitope of CD38. Such a nucleic acid sequence may encode a variable heavy chain of an antibody and include a sequence selected from the group consisting of SEQ ID NOS: 1, 2, 3, or 4, or a nucleic acid sequence that hybridizes under high stringency conditions to the complimentary strand of SEQ ID NO: 1, 2, 3, or 4. The nucleic acid might
25 encode a variable light chain of an isolated antibody or functional fragment thereof, and may contains a sequence selected from the group consisting of SEQ

ID NOS: 9, 10, 11, or 12 , or a nucleic acid sequence that hybridizes under high stringency conditions to the complimentary strand of SEQ ID NO: 9, 10, 11, or 12.

Nucleic acids of the invention are suitable for recombinant production.

5 Thus, the invention also relates to vectors and host cells containing a nucleic acid sequence of the invention.

Compositions of the invention may be used for therapeutic or prophylactic applications. The invention, therefore, includes a pharmaceutical composition containing an inventive antibody (or functional antibody fragment) and a
10 pharmaceutically acceptable carrier or excipient therefor. In a related aspect, the invention provides a method for treating a disorder or condition associated with the undesired presence of CD38. Such method contains the steps of administering to a subject in need thereof an effective amount of the pharmaceutical composition that contains an inventive antibody as described or contemplated
15 herein.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1a provides nucleic acid sequences of various novel antibody
20 variable heavy regions.

Figure 1b provides amino acid sequences of various novel antibody variable heavy regions. CDR regions HCDR1, HCDR2 and HCDR3 are designated from N- to C-terminus in boldface.

Figure 2a provides nucleic acid sequences of various novel antibody
25 variable light regions.

Figure 2b provides amino acid sequences of various novel antibody variable light regions. CDR regions LCDR1, LCDR2 and LCDR3 are designated from N- to C-terminus in boldface.

Figure 3 provides amino acid sequences of variable heavy regions of various consensus-based HuCAL antibody master gene sequences. CDR regions HCDR1, HCDR2 and HCDR3 are designated from N- to C-terminus in boldface.

Figure 4 provides amino acid sequences of variable light regions of various consensus-based HuCAL antibody master gene sequences. CDR regions LCDR1, LCDR2 and LCDR3 are designated from N- to C-terminus in boldface.

Figure 5 provides the amino acid sequence of CD38 (SWISS-PROT primary accession number P28907).

Figure 6 provides the nucleotide sequences of the heavy and light chains of chimeric OKT10.

Figure 7 provides a schematic overview of epitopes of representative antibodies of the present invention.

Figure 8 provides the DNA sequence of pMORPH@_h_IgG1_1 (bp 601-2200) (SEQ ID NO: 32): The vector is based on the pcDNA3.1+ vectors (Invitrogen). The amino acid sequence of the VH-stuffer sequence is indicated in red, whereas the final reading frames of the VH-leader sequence and the constant region gene are printed in black. Restriction sites are indicated above the sequence. The priming sites of the sequencing primers are underlined.

Figure 9 provides the DNA sequence of Ig kappa light chain expression vector pMORPH@_h_Igκ_1 (bp 601-1400) (SEQ ID NO: 33): The vector is based on the pcDNA3.1+ vectors (Invitrogen). The amino acid sequences of the Vκ-stuffer sequence is indicated in red, whereas the final reading frames of the

V κ -leader sequence and of the constant region gene are printed in black.

Restriction sites are indicated above the sequence. The priming sites of the sequencing primers are underlined.

Figure 10 provides the DNA sequence of HuCAL Ig lambda light chain
5 vector pMORPH@_h_Ig λ _1 (bp 601-1400) (SEQ ID NO: 34): The amino acid
sequence of the V λ -stuffer sequence is indicated in red, whereas the final reading
frames of the V λ -leader sequence and of the constant region gene are printed in
black. Restriction sites are indicated above the sequence. The priming sites of the
sequencing primers are underlined.

10

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the discovery of novel human antibodies
that are specific to or have a high affinity for CD38 and can deliver a therapeutic
benefit to a subject. The antibodies of the invention can be used in many
15 contexts, which are more fully described herein.

A "human" antibody or functional antibody fragment is hereby defined as
one that not chimeric (e.g., not "humanized") and not from (either in whole or in
part) a non-human species. A human antibody or functional antibody fragment
can be derived directly from a human or can be derived at least partially *in silico*
20 from synthetic sequences that are based on the analysis of known human antibody
sequences.

As used herein, an antibody "binds specifically to," is "specific to/for" or
"specifically recognizes" an antigen (here, CD38) if such antibody is able to
discriminate between such antigen and one or more reference antigen(s), since
25 binding specificity is not an absolute, but a relative property. In its most general

form (and when no defined reference is mentioned), "specific binding" is referring to the ability of the antibody to discriminate between the antigen of interest and an unrelated antigen, as determined, for example, in accordance with one of the following methods. Such methods comprise, but are not limited to Western blots, ELISA-, RIA-, ECL-, IRMA-tests and peptide scans. For example, a standard ELISA assay can be carried out. The scoring may be carried out by standard color development (e.g. secondary antibody with horseradish peroxidase and tetramethyl benzidine with hydrogenperoxide). The reaction in certain wells is scored by the optical density, for example, at 450 nm. Typical background (=negative reaction) may be 0.1 OD; typical positive reaction may be 1 OD. This means the difference positive/negative can be more than 10-fold. Typically, determination of binding specificity is performed by using not a single reference antigen, but a set of about three to five unrelated antigens, such as milk powder, BSA, transferrin or the like.

However, "specific binding" also may refer to the ability of an antibody to discriminate between the target antigen and one or more closely related antigen(s), which are used as reference points, e.g. between CD38 and CD157. Additionally, "specific binding" may relate to the ability of an antibody to discriminate between different parts of its target antigen, e.g. different domains or regions of CD38, such as epitopes in the N-terminal or in the C-terminal region of CD38, or between one or more key amino acid residues or stretches of amino acid residues of CD38.

Also, as used herein, an "immunoglobulin" (Ig) hereby is defined as a protein belonging to the class IgG, IgM, IgE, IgA, or IgD (or any subclass thereof), and includes all conventionally known antibodies and functional fragments thereof. A "functional fragment" of an antibody/immunoglobulin hereby is defined as a fragment of an antibody/immunoglobulin (e.g., a variable

region of an IgG) that retains the antigen-binding region. An “antigen-binding region” of an antibody typically is found in one or more hypervariable region(s) of an antibody, *i.e.*, the CDR-1, -2, and/or -3 regions; however, the variable “framework” regions can also play an important role in antigen binding, such as by providing a scaffold for the CDRs. Preferably, the “antigen-binding region” comprises at least amino acid residues 4 to 103 of the variable light (VL) chain and 5 to 109 of the variable heavy (VH) chain, more preferably amino acid residues 3 to 107 of VL and 4 to 111 of VH, and particularly preferred are the complete VL and VH chains (amino acid positions 1 to 109 of VL and 1 to 113 of VH; numbering according to WO 97/08320). A preferred class of immunoglobulins for use in the present invention is IgG. “Functional fragments” of the invention include the domain of a F(ab')₂ fragment, a Fab fragment and scFv. The F(ab')₂ or Fab may be engineered to minimize or completely remove the intermolecular disulphide interactions that occur between the C_{H1} and C_L domains.

An antibody of the invention may be derived from a recombinant antibody library that is based on amino acid sequences that have been designed *in silico* and encoded by nucleic acids that are synthetically created. *In silico* design of an antibody sequence is achieved, for example, by analyzing a database of human sequences and devising a polypeptide sequence utilizing the data obtained therefrom. Methods for designing and obtaining *in silico*-created sequences are described, for example, in Knappik *et al.*, J. Mol. Biol. (2000) 296:55; Krebs *et al.*, J. Immunol. Methods. (2001) 254:67; and U.S. Patent No. 6,300,064 issued to Knappik *et al.*, which hereby are incorporated by reference in their entirety.

Antibodies of the Invention

Throughout this document, reference is made to the following representative antibodies of the invention: "antibody nos." or "LACS" 3077, 3079, 3080 and 3100. LAC 3077 represents an antibody having a variable heavy region
5 corresponding to SEQ ID NO: 1 (DNA)/SEQ ID NO: 5 (protein) and a variable light region corresponding to SEQ ID NO: 9 (DNA)/SEQ ID NO: 13 (protein). LAC 3079 represents an antibody having a variable heavy region corresponding to SEQ ID NO: 2 (DNA)/SEQ ID NO: 6 (protein) and a variable light region corresponding to SEQ ID NO: 10 (DNA)/SEQ ID NO: 14 (protein). LAC 3080
10 represents an antibody having a variable heavy region corresponding to SEQ ID NO: 3 (DNA)/SEQ ID NO: 7 (protein) and a variable light region corresponding to SEQ ID NO: 11 (DNA)/SEQ ID NO: 15 (protein). LAC 3100 represents an antibody having a variable heavy region corresponding to SEQ ID NO: 4 (DNA)/SEQ ID NO: 8 (protein) and a variable light region corresponding to SEQ
15 ID NO: 12 (DNA)/SEQ ID NO: 16 (protein).

In one aspect, the invention provides antibodies having an antigen-binding region that can bind specifically to or has a high affinity for one or more regions of CD38, whose amino acid sequence is depicted by SEQ ID NO: 22. An antibody is said to have a "high affinity" for an antigen if the affinity
20 measurement is at least 100 nM (monovalent affinity of Fab fragment). An inventive antibody or antigen-binding region preferably can bind to CD38 with an affinity of about less than 100 nM, more preferably less than about 60 nM, and still more preferably less than about 30 nM. Further preferred are antibodies that bind to CD38 with an affinity of less than about 10 nM, and more preferably less
25 than 3 about nM. For instance, the affinity of an antibody of the invention against CD38 may be about 10.0 nM or 2.4 nM (monovalent affinity of Fab fragment).

Table 1 provides a summary of affinities of representative antibodies of the invention, as determined by surface plasmon resonance (Biacore) and FACS Scatchard analysis:

5 **Table 1: Antibody Affinities**

Antibody (Fab or IgG1)	BIACORE (Fab)	FACS Scatchard (IgG1)
	Kd [nM]	Kd [nM]
MOR03077	56.0	2.1
MOR03079	2.4	2.3
MOR03080	27.5	2.3
MOR03100	10.0	22.5
Chimeric OKT10	Not determined (Fab fragment not available)	56.0

With reference to Table 1, the affinity of LACs 3077, 3079, 3080 and 3100 was measured by surface plasmon resonance (Biacore) on immobilized recombinant CD38 and by a flow cytometry procedure utilizing the CD38-expressing human RPMI8226 cell line. The Biacore studies were performed on directly immobilized antigen (CD38-Fc fusion protein). The Fab format of LACs 3077, 3079, 3080 and 3100 exhibit a monovalent affinity range between about 2.4 and 56 nM on immobilized CD38-Fc fusion protein with LAC 3079 showing the highest affinity, followed by Fabs 3100, 3080 and 3077.

10

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The IgG1 format was used for the cell-based affinity determination (FACS Scatchard). The right column of Table 1 denotes the binding strength of the LACS in this format. LAC 3077 showed the strongest binding, which is slightly stronger than LACS 3080 and 3079.

5 Another feature of preferred antibodies of the invention is their specificity for an area within the N-terminal region of CD38. The N-terminal region is made up of amino acids 44 to 224 of CD38. All representative LACs 3077, 3079, 3080, and 3100 of the invention can bind specifically to the N-terminal region of CD38.

The type of epitope to which an antibody of the invention binds may be
10 linear (i.e. one consecutive stretch of amino acids) or conformational (i.e. multiple stretches of amino acids). In order to determine whether the epitope of a particular antibody is linear or conformational, the skilled work can analyze the binding of antibodies to overlapping peptides (e.g., 13-mer peptides with an overlap of 11 amino acids) covering different domains of CD38. Using this
15 analysis, the inventors have discovered that LACS 3077, 3080, and 3100 recognize discontinuous epitopes in the N-terminal region of CD38, whereas the epitope of LAC 3079 can be described as linear (see Figure 7).

An antibody of the invention preferably is species cross-reactive with humans and at least one other species, which may be a rodent species or a non-
20 human primate. The non-human primate can be rhesus, baboon and/or cynomolgus. The rodent species can be mouse, rat and/or hamster. An antibody that is cross reactive with at least one rodent species, for example, can provide greater flexibility and benefits over known anti-CD38 antibodies, for purposes of conducting *in vivo* studies in multiple species with the same antibody.

Preferably, an antibody of the invention not only is able to bind to CD38, but also is able to mediate killing of a cell expressing CD38. More specifically, an antibody of the invention can mediate its therapeutic effect by depleting CD38-positive (*e.g.*, malignant) cells via antibody-effector functions. These functions include antibody-dependent cellular cytotoxicity (ADCC) and complement mediated cytotoxicity (CDC).

Table 2 provides a summary of the determination of EC₅₀ values of representative antibodies of the invention in both ADCC and CDC:

Table 2: EC₅₀ Values of Antibodies

Antibody (IgG1)	ADCC		CDC
	EC ₅₀ [nM]		EC ₅₀ [nM]
	LP-1	RPMI8226	CHO-transfectants
MOR03077	0.60 ^a	0.08	0.8 ^a
MOR03079	0.09 ^a	0.04	0.40 ^a
MOR03080	0.17 ^b	0.05	3.2 ^a
MOR03100	0.28 ^b	0.28	15.7 ^a
Chimeric OKT10	5.23 ^a	4.10	9.3 ^a

^a: mean from at least 2 EC₅₀ determinations

^b: single determination

Peptide Variants

Antibodies of the invention are not limited to the specific peptide sequences provided herein. Rather, the invention also embodies variants of these polypeptides. With reference to the instant disclosure and conventionally

available technologies and references, the skilled worker will be able to prepare, test and utilize functional variants of the antibodies disclosed herein, while appreciating that variants having the ability to mediate killing of a CD38+ target cell fall within the scope of the present invention. As used in this context, “ability to mediate killing of a CD38+ target cell” means a functional characteristic ascribed to an anti-CD38 antibody of the invention. Ability to mediate killing of a CD38+ target cell, thus, includes the ability to mediate killing of a CD38+ target cell, *e.g.*, by ADCC and/or CDC, or by toxin constructs conjugated to an antibody of the invention.

10 A variant can include, for example, an antibody that has at least one altered complementarity determining region (CDR) (hyper-variable) and/or framework (FR) (variable) domain/position, vis-à-vis a peptide sequence disclosed herein. To better illustrate this concept, a brief description of antibody structure follows.

An antibody is composed of two peptide chains, each containing one (light chain) or three (heavy chain) constant domains and a variable region (VL, VH), the latter of which is in each case made up of four FR regions and three interspaced CDRs. The antigen-binding site is formed by one or more CDRs, yet the FR regions provide the structural framework for the CDRs and, hence, play an important role in antigen binding. By altering one or more amino acid residues in a CDR or FR region, the skilled worker routinely can generate mutated or diversified antibody sequences, which can be screened against the antigen, for new or improved properties, for example.

20 Tables 3a (VH) and 3b (VL) delineate the CDR and FR regions for certain antibodies of the invention and compare amino acids at a given position to each other and to corresponding consensus or “master gene” sequences (as described in U.S. Patent No. 6,300,064):

Table 3a: VH Sequences

VH

VH sequences CD38 binders

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Table 3a: VH Sequences

VH

Position	Framework 3										CDR3										Framework 4																					
	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3					
SEQ ID NO: 3077	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3				
	BSeq1																				Sv1										BSeq1											
	R	V	T	M	T	R	D	T	S	I	S	T	A	Y	M	E	L	S	S	L	R	S	E	D	T	A	V	Y	C	A	R	W	G	Q	G	T	L	V	T	V	S	S
	R	V	T	M	T	R	D	T	S	I	S	T	A	Y	M	E	L	S	S	L	R	S	E	D	T	A	V	Y	C	A	R	W	G	Q	G	T	L	V	T	V	S	S
SEQ ID NO: 3079	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3				
	BSeq1																				Sv1										BSeq1											
	R	F	T	I	S	R	D	N	S	K	N	T	L	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	C	A	R	W	G	Q	G	T	L	V	T	V	S	S
	R	F	T	I	S	R	D	N	S	K	N	T	L	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	C	A	R	W	G	Q	G	T	L	V	T	V	S	S
SEQ ID NO: 3080	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3				
	BSeq1																				Sv1										BSeq1											
	R	F	T	I	S	R	D	N	S	K	N	T	L	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	C	A	R	W	G	Q	G	T	L	V	T	V	S	S
	R	F	T	I	S	R	D	N	S	K	N	T	L	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	C	A	R	W	G	Q	G	T	L	V	T	V	S	S
SEQ ID NO: 3100	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3				
	BSeq1																				Sv1										BSeq1											
	R	F	T	I	S	R	D	N	S	K	N	T	L	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	C	A	R	W	G	Q	G	T	L	V	T	V	S	S
	R	F	T	I	S	R	D	N	S	K	N	T	L	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	C	A	R	W	G	Q	G	T	L	V	T	V	S	S

Table 3b: VL Sequences

VL sequences CD38 binders																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
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VL13	SEQ ID NO 21	S	V	E	L	T	Q	P	P	-	S	V	S	V	A	P	G	Q	T	A	R	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A	S	I	S	C	S	S	G	D	A

Table 3b: VL Sequences

		Framework 3										CDR3										Framework 4																					
		7										8										9										10											
Position		5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9							
	VL1	S	N	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	D	H	A	T	T	V	F	G	G	G	T	K	L	T	V	L	G
	3080	S	N	S	G	N	T	A	T	L	T	I	S	G	T	Q	A	E	D	E	A	D	Y	Y	C	Q	D	H	A	T	T	V	F	G	G	G	T	K	L	T	V	L	G
	3100	S	N	S	G	N	T	A	T	L	T	I	S	G	T	Q	A	E	D	E	A	D	Y	Y	C	Q	D	H	A	T	T	V	F	G	G	G	T	K	L	T	V	L	G
	VL1	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	D	H	A	T	T	V	F	G	G	G	T	K	V	E	I	K	R
	3079	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	D	H	A	T	T	V	F	G	G	G	T	K	V	E	I	K	R
	VL2	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	D	H	A	T	T	V	F	G	G	G	T	K	L	E	I	K	R
	3077	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	D	H	A	T	T	V	F	G	G	G	T	K	V	E	I	K	R

The skilled worker can use the data in Tables 3a and 3b to design peptide variants that are within the scope of the present invention. It is preferred that variants are constructed by changing amino acids within one or more CDR regions; a variant might also have one or more altered framework regions. With
5 reference to a comparison of the novel antibodies to each other, candidate residues that can be changed include e.g. residues 4 or 37 of the variable light and e.g. residues 13 or 43 of the variable heavy chains of LAC's 3080 and 3077, since these are positions of variance vis-à-vis each other. Alterations also may be made in the framework regions. For example, a peptide FR domain might be altered
10 where there is a deviation in a residue compared to a germline sequence.

With reference to a comparison of the novel antibodies to the corresponding consensus or "master gene" sequence, candidate residues that can be changed include e.g. residues 27, 50 or 90 of the variable light chain of LAC 3080 compared to VL λ 3 and e.g. residues 33, 52 and 97 of the variable heavy chain of
15 LAC 3080 compared to VH3. Alternatively, the skilled worker could make the same analysis by comparing the amino acid sequences disclosed herein to known sequences of the same class of such antibodies, using, for example, the procedure described by Knappik *et al.*, *supra* and U.S. Patent No. 6,300,064 issued to Knappik *et al.*

20 Furthermore, variants may be obtained by using one LAC as starting point for optimization by diversifying one or more amino acid residues in the LAC, preferably amino acid residues in one or more CDRs, and by screening the resulting collection of antibody variants for variants with improved properties. Particularly preferred is diversification of one or more amino acid residues in
25 CDR-3 of VL, CDR-3 of VH, CDR-1 of VL and/or CDR-2 of VH.

Diversification can be done by synthesizing a collection of DNA molecules using trinucleotide mutagenesis (TRIM) technology (Virnekäs *et al.*, 1994).

Conservative Amino Acid Variants

Polypeptide variants may be made that conserve the overall molecular structure of an antibody peptide sequence described herein. Given the properties of the individual amino acids, some rational substitutions will be recognized by the skilled worker. Amino acid substitutions, *i.e.*, "conservative substitutions," may be made, for instance, on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved.

For example, (a) nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; (b) polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; (c) positively charged (basic) amino acids include arginine, lysine, and histidine; and (d) negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Substitutions typically may be made within groups (a)-(d). In addition, glycine and proline may be substituted for one another based on their ability to disrupt α -helices. Similarly, certain amino acids, such as alanine, cysteine, leucine, methionine, glutamic acid, glutamine, histidine and lysine are more commonly found in α -helices, while valine, isoleucine, phenylalanine, tyrosine, tryptophan and threonine are more commonly found in β -pleated sheets. Glycine, serine, aspartic acid, asparagine, and proline are commonly found in turns. Some preferred substitutions may be made among the following groups: (i) S and T; (ii) P and G; and (iii) A, V, L and I. Given the known genetic code, and recombinant and synthetic DNA techniques, the skilled scientist readily can construct DNAs encoding the conservative amino acid

variants. In one particular example, amino acid position 3 in SEQ ID NOS: 5, 6, 7, and/or 8 can be changed from a Q to an E.

As used herein, "sequence identity" between two polypeptide sequences indicates the percentage of amino acids that are identical between the sequences.

5 "Sequence similarity" indicates the percentage of amino acids that either are identical or that represent conservative amino acid substitutions. Preferred polypeptide sequences of the invention have a sequence identity in the CDR regions of at least 60%, more preferably, at least 70% or 80%, still more preferably at least 90% and most preferably at least 95%. Preferred antibodies

10 also have a sequence similarity in the CDR regions of at least 80%, more preferably 90% and most preferably 95%.

DNA molecules of the invention

The present invention also relates to the DNA molecules that encode an antibody of the invention. These sequences include, but are not limited to, those

15 DNA molecules set forth in Figures 1a and 2a.

DNA molecules of the invention are not limited to the sequences disclosed herein, but also include variants thereof. DNA variants within the invention may be described by reference to their physical properties in hybridization. The skilled worker will recognize that DNA can be used to identify its complement and, since

20 DNA is double stranded, its equivalent or homolog, using nucleic acid hybridization techniques. It also will be recognized that hybridization can occur with less than 100% complementarity. However, given appropriate choice of conditions, hybridization techniques can be used to differentiate among DNA sequences based on their structural relatedness to a particular probe. For guidance

25 regarding such conditions see, Sambrook *et al.*, 1989 and Ausubel *et al.*, 1989.

Structural similarity between two polynucleotide sequences can be expressed as a function of "stringency" of the conditions under which the two sequences will hybridize with one another. As used herein, the term "stringency" refers to the extent that the conditions disfavor hybridization. Stringent conditions
5 strongly disfavor hybridization, and only the most structurally related molecules will hybridize to one another under such conditions. Conversely, non-stringent conditions favor hybridization of molecules displaying a lesser degree of structural relatedness. Hybridization stringency, therefore, directly correlates with the structural relationships of two nucleic acid sequences. The following
10 relationships are useful in correlating hybridization and relatedness (where T_m is the melting temperature of a nucleic acid duplex):

- a. $T_m = 69.3 + 0.41(G+C)\%$
- b. The T_m of a duplex DNA decreases by 1°C with every
15 increase of 1% in the number of mismatched base pairs.
- c. $(T_m)_{\mu 2} - (T_m)_{\mu 1} = 18.5 \log_{10} \mu 2 / \mu 1$
where $\mu 1$ and $\mu 2$ are the ionic strengths of two solutions.

20 Hybridization stringency is a function of many factors, including overall DNA concentration, ionic strength, temperature, probe size and the presence of agents which disrupt hydrogen bonding. Factors promoting hybridization include high DNA concentrations, high ionic strengths, low temperatures, longer probe size and the absence of agents that disrupt hydrogen bonding. Hybridization
25 typically is performed in two phases: the "binding" phase and the "washing" phase.

First, in the binding phase, the probe is bound to the target under conditions favoring hybridization. Stringency is usually controlled at this stage by altering the temperature. For high stringency, the temperature is usually between 65°C

and 70°C, unless short (< 20 nt) oligonucleotide probes are used. A representative hybridization solution comprises 6X SSC, 0.5% SDS, 5X Denhardt's solution and 100 µg of nonspecific carrier DNA. See Ausubel *et al.*, *supra*, section 2.9, supplement 27 (1994). Of course, many different, yet functionally equivalent, 5 buffer conditions are known. Where the degree of relatedness is lower, a lower temperature may be chosen. Low stringency binding temperatures are between about 25°C and 40°C. Medium stringency is between at least about 40°C to less than about 65°C. High stringency is at least about 65°C.

Second, the excess probe is removed by washing. It is at this phase that 10 more stringent conditions usually are applied. Hence, it is this "washing" stage that is most important in determining relatedness via hybridization. Washing solutions typically contain lower salt concentrations. One exemplary medium stringency solution contains 2X SSC and 0.1% SDS. A high stringency wash solution contains the equivalent (in ionic strength) of less than about 0.2X SSC, 15 with a preferred stringent solution containing about 0.1X SSC. The temperatures associated with various stringencies are the same as discussed above for "binding." The washing solution also typically is replaced a number of times during washing. For example, typical high stringency washing conditions comprise washing twice for 30 minutes at 55° C. and three times for 15 minutes at 20 60° C.

The present invention includes nucleic acid molecules that hybridize to the molecules of set forth in Figures 1a and 2a under high stringency binding and washing conditions, where such nucleic molecules encode an antibody or functional fragment thereof having properties as described herein. Preferred 25 molecules (from an mRNA perspective) are those that have at least 75% or 80% (preferably at least 85%, more preferably at least 90% and most preferably at least

95%) homology or sequence identity with one of the DNA molecules described herein. In one particular example of a variant of the invention, nucleic acid position 7 in SEQ ID NOS: 1, 2, 3 and/or 4 can be substituted from a C to an G, thereby changing the codon from CAA to GAA.

5 ***Functionally Equivalent Variants***

Yet another class of DNA variants within the scope of the invention may be described with reference to the product they encode (see the peptides listed in figures 1b and 2b). These functionally equivalent genes characterized by the fact that they encode the same peptide sequences found in figures 1b and 2b due to the
10 degeneracy of the genetic code. SEQ ID NOS: 1 and 31 are an example of functionally equivalent variants, as their nucleic acid sequences are different, yet they encode the same polypeptide, i.e. SEQ ID NO: 5.

It is recognized that variants of DNA molecules provided herein can be constructed in several different ways. For example, they may be constructed as
15 completely synthetic DNAs. Methods of efficiently synthesizing oligonucleotides in the range of 20 to about 150 nucleotides are widely available. See Ausubel *et al.*, *supra*, section 2.11, Supplement 21 (1993). Overlapping oligonucleotides may be synthesized and assembled in a fashion first reported by Khorana *et al.*, J. Mol. Biol. 72:209-217 (1971); see also Ausubel *et al.*, *supra*, Section 8.2.
20 Synthetic DNAs preferably are designed with convenient restriction sites engineered at the 5' and 3' ends of the gene to facilitate cloning into an appropriate vector.

As indicated, a method of generating variants is to start with one of the DNAs disclosed herein and then to conduct site-directed mutagenesis. See
25 Ausubel *et al.*, *supra*, chapter 8, Supplement 37 (1997). In a typical method, a target DNA is cloned into a single-stranded DNA bacteriophage vehicle. Single-

stranded DNA is isolated and hybridized with a oligonucleotide containing the desired nucleotide alteration(s). The complementary strand is synthesized and the double stranded phage is introduced into a host. Some of the resulting progeny will contain the desired mutant, which can be confirmed using DNA sequencing.

5 In addition, various methods are available that increase the probability that the progeny phage will be the desired mutant. These methods are well known to those in the field and kits are commercially available for generating such mutants.

Recombinant DNA constructs and expression

The present invention further provides recombinant DNA constructs
10 comprising one or more of the nucleotide sequences of the present invention. The recombinant constructs of the present invention are used in connection with a vector, such as a plasmid or viral vector, into which a DNA molecule encoding an antibody of the invention is inserted.

The encoded gene may be produced by techniques described in Sambrook *et al.*, 1989, and Ausubel *et al.*, 1989. Alternatively, the DNA sequences may be
15 chemically synthesized using, for example, synthesizers. See, for example, the techniques described in OLIGONUCLEOTIDE SYNTHESIS (1984, Gait, ed., IRL Press, Oxford), which is incorporated by reference herein in its entirety. Recombinant constructs of the invention are comprised with expression vectors
20 that are capable of expressing the RNA and/or protein products of the encoded DNA(s). The vector may further comprise regulatory sequences, including a promoter operably linked to the open reading frame (ORF). The vector may further comprise a selectable marker sequence. Specific initiation and bacterial secretory signals also may be required for efficient translation of inserted target
25 gene coding sequences.

The present invention further provides host cells containing at least one of the DNAs of the present invention. The host cell can be virtually any cell for which expression vectors are available. It may be, for example, a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, but preferably is a prokaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran mediated transfection, electroporation or phage infection.

Bacterial Expression

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and, if desirable, to provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*.

Bacterial vectors may be, for example, bacteriophage-, plasmid- or phagemid-based. These vectors can contain a selectable marker and bacterial origin of replication derived from commercially available plasmids typically containing elements of the well known cloning vector pBR322 (ATCC 37017). Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is de-repressed/induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation,

disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the protein being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of antibodies or to screen peptide libraries, for example, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable.

10 Therapeutic Methods

Therapeutic methods involve administering to a subject in need of treatment a therapeutically effective amount of an antibody contemplated by the invention. A "therapeutically effective" amount hereby is defined as the amount of an antibody that is of sufficient quantity to deplete CD38-positive cells in a treated area of a subject—either as a single dose or according to a multiple dose regimen, alone or in combination with other agents, which leads to the alleviation of an adverse condition, yet which amount is toxicologically tolerable. The subject may be a human or non-human animal (*e.g.*, rabbit, rat, mouse, monkey or other lower-order primate).

An antibody of the invention might be co-administered with known medicaments, and in some instances the antibody might itself be modified. For example, an antibody could be conjugated to an immunotoxin or radioisotope to potentially further increase efficacy.

The inventive antibodies can be used as a therapeutic or a diagnostic tool in a variety of situations where CD38 is undesirably expressed or found. Disorders

and conditions particularly suitable for treatment with an antibody of the inventions are multiple myeloma (MM) and other haematological diseases, such as chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), acute myelogenous leukemia (AML), and acute lymphocytic leukemia (ALL). An
5 antibody of the invention also might be used to treat inflammatory disease such as rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE).

To treat any of the foregoing disorders, pharmaceutical compositions for use in accordance with the present invention may be formulated in a conventional manner using one or more physiologically acceptable carriers or excipients. An
10 antibody of the invention can be administered by any suitable means, which can vary, depending on the type of disorder being treated. Possible administration routes include parenteral (*e.g.*, intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous), intrapulmonary and intranasal, and, if desired for local immunosuppressive treatment, intralesional administration. In addition,
15 an antibody of the invention might be administered by pulse infusion, with, *e.g.*, declining doses of the antibody. Preferably, the dosing is given by injections, most preferably intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. The amount to be administered will depend on a variety of factors such as the clinical symptoms, weight of the individual,
20 whether other drugs are administered. The skilled artisan will recognize that the route of administration will vary depending on the disorder or condition to be treated.

Determining a therapeutically effective amount of the novel polypeptide, according to this invention, largely will depend on particular patient
25 characteristics, route of administration, and the nature of the disorder being treated. General guidance can be found, for example, in the publications of the

International Conference on Harmonisation and in REMINGTON'S PHARMACEUTICAL SCIENCES, chapters 27 and 28, pp. 484-528 (18th ed., Alfonso R. Gennaro, Ed., Easton, Pa.: Mack Pub. Co., 1990). More specifically, determining a therapeutically effective amount will depend on such factors as toxicity and efficacy of the medicament. Toxicity may be determined using methods well known in the art and found in the foregoing references. Efficacy may be determined utilizing the same guidance in conjunction with the methods described below in the Examples.

Diagnostic Methods

CD38 is highly expressed on hematological cells in certain malignancies; thus, an anti-CD38 antibody of the invention may be employed in order to image or visualize a site of possible accumulation of malignant cells in a patient. In this regard, an antibody can be detectably labeled, through the use of radioisotopes, affinity labels (such as biotin, avidin, etc.) fluorescent labels, paramagnetic atoms, etc. Procedures for accomplishing such labeling are well known to the art. Clinical application of antibodies in diagnostic imaging are reviewed by Grossman, H. B., Urol. Clin. North Amer. 13:465-474 (1986)), Unger, E. C. et al., Invest. Radiol. 20:693-700 (1985)), and Khaw, B. A. et al., Science 209:295-297 (1980)).

The detection of foci of such detectably labeled antibodies might be indicative of a site of tumor development, for example. In one embodiment, this examination is done by removing samples of tissue or blood and incubating such samples in the presence of the detectably labeled antibodies. In a preferred embodiment, this technique is done in a non-invasive manner through the use of magnetic imaging, fluorography, etc. Such a diagnostic test may be employed in

monitoring the success of treatment of diseases, where presence or absence of CD38-positive cells is a relevant indicator.

Therapeutic And Diagnostic Compositions

5 The antibodies the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, wherein an antibody of the invention (including any functional fragment thereof) is combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation are described, for example, in REMINGTON'S
10 PHARMACEUTICAL SCIENCES (18th ed., Alfonso R. Gennaro, Ed., Easton, Pa.: Mack Pub. Co., 1990). In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of one or more of the antibodies of the present invention, together with a suitable amount of carrier vehicle.

15 Preparations may be suitably formulated to give controlled-release of the active compound. Controlled-release preparations may be achieved through the use of polymers to complex or absorb anti-CD38 antibody. The controlled delivery may be exercised by selecting appropriate macromolecules (for example polyesters, polyamino acids, polyvinyl, pyrrolidone, ethylenevinyl-acetate,
20 methylcellulose, carboxymethylcellulose, or protamine, sulfate) and the concentration of macromolecules as well as the methods of incorporation in order to control release. Another possible method to control the duration of action by controlled release preparations is to incorporate anti-CD38 antibody into particles of a polymeric material such as polyesters, polyamino acids, hydrogels,
25 poly(lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating these agents into polymeric particles, it is possible to entrap these

materials in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatine-microcapsules and poly(methylmethacrylate) microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, 5 microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (1980).

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampules or in multi-dose 10 containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

15 The compositions may, if desired, be presented in a pack or dispenser device, which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

20 The invention further is understood by reference to the following working examples, which are intended to illustrate and, hence, not limit the invention.

EXAMPLES

Cell-lines

The following cell-lines were obtained from the European Collection of Cell Cultures (ECACC); the German German Collection of Microorganisms (DSMZ) or the American Type Culture collection (ATCC): hybridoma cell line producing the CD38 mouse IgG1 monoclonal antibody OKT10 (ECACC, #87021903), Jurkat cells (DSMZ, ACC282), LP-1 (DSMZ, ACC41); RPMI (ATCC, CCL-155); HEK293 (ATCC, CRL-1573), CHO-K1 (ATCC, CRL-61), Raji (ATCC, CCL-86)

10

Cells and culture-conditions

All cells were cultured under standardized conditions at 37°C and 5% CO₂ in a humidified incubator. The cell-lines LP-1, RPMI8226, Jurkat and Raji were cultured in RPMI1640 (Pan biotech GmbH, #P04-16500) supplemented with 10 % FCS (PAN biotech GmbH, #P30-3302), 50 U/ml penicillin, 50 µg/ml streptomycin (Gibco, #15140-122) and 2 mM glutamine (Gibco, #25030-024) and, in case of Jurkat- and Raji-cells, additionally 10 mM Hepes (Pan biothech, #P05-01100) and 1 mM sodium pyruvate (Pan biotech, # P04-43100) had to be added.

EL4, CHO and HEK293 were grown in DMEM (Gibco, #10938-025) supplemented with 2 mM glutamine and 10% FCS. Stable CD38 CHO-transfectants were maintained in the presence of G418 (PAA GmbH, P11-012) whereas for HEK293 the addition of 1mM sodium-pyruvate was essential. After transient transfection of HEK293 the 10% FCS was replaced by Ultra low IgG FCS (Invitrogen, #16250-078). The cell-line OKT10 was cultured in IDMEM (Gibco, #31980-022), supplemented with 2 mM glutamine and 20 % FCS.

25

Preparation of single cell suspensions from peripheral blood:

All blood samples were taken after informed consent. Peripheral blood mononuclear cells (PBMC) were isolated by Histopaque®-1077 (Sigma) according to the manufacturer's instructions from healthy donors. Red blood cells were depleted from these cell suspensions by incubation in ACK Lysis Buffer (0.15 M NH₄Cl, 10 mM KHCO₃, 0.1 M EDTA) for 5 min at RT or a commercial

30

derivative (Bioscience, #00-4333). Cells were washed twice with PBS and then further processed for flow cytometry or ADCC (see below).

Flow cytometry ("FACS")

- 5 All stainings were performed in round bottom 96-well culture plates (Nalge Nunc) with 2×10^5 cells per well. Cells were incubated with Fabs or IgG antibodies at the indicated concentrations in 50 μ l FACS buffer (PBS, 3% FCS, 0.02% NaN₃) for 40 min at 4°C. Cells were washed twice and then incubated with R-Phycoerythrin (PE) conjugated goat-anti-human or goat-anti-mouse IgG (H+L) F(ab')₂ (Jackson Immuno Research), diluted 1:200 in FACS buffer, for 30 min at 10 4°C. Cells were again washed, resuspended in 0.3 ml FACS buffer and then analyzed by flow cytometry in a FACSCalibur (Becton Dickinson, San Diego, CA).

- For FACS based Scatchard analyses RPMI8226 cells were stained with at 12 different dilutions (1:2n) starting at 50 μ g/ml (Fab) or 10 μ g/ml (IgG) final 15 concentration. At least two independent measurements were used for each concentrations and K_d values extrapolated from median fluorescence intensities according to Chamow et al. (1994).

20 Surface plasmon resonance

- The kinetic constants k_{on} and k_{off} were determined with serial dilutions of the respective Fab binding to covalently immobilized CD38-Fc fusion protein using the BIAcore 3000 instrument (Biacore, Uppsala, Sweden). For covalent antigen immobilization standard EDC-NHS amine coupling chemistry was used. For 25 direct coupling of CD38 Fc-fusion protein (R&D) a F1 sensor chips (Biacore) were coated with ~600-700 RU in 10 mM acetate buffer, pH 4.5. For the reference flow cell a respective amount of HSA was used. Kinetic measurements were done in PBS (136 mM NaCl, 2.7 mM KCl, 10mM Na₂HPO₄, 1,76 mM KH₂PO₄ pH 7.4) at a flow rate of 20 μ l/min using Fab concentration range from 1.5-500 nM. 30 Injection time for each concentration was 1 min, followed by 3 min dissociation phase. For regeneration 5 μ l 10mM HCl was used. All sensograms were fitted globally using BIA evaluation software 3.1 (Biacore).

EXAMPLE 1: Antibody Generation from HuCAL Libraries

For the generation of therapeutic antibodies against CD38, selections with the MorphoSys HuCAL® GOLD phage display library were carried out. HuCAL® GOLD is a Fab library based on the HuCAL® concept (Knappik et al., 2000; Krebs et al., 2001), in which all six CDRs are diversified, and which employs the CysDisplay™ technology for linking Fab fragments to the phage surface (Löhning, 2001).

A. Phagemid rescue, phage amplification and purification

HuCAL® GOLD phagemid library was amplified in 2 x TY medium containing 34 µg/ml chloramphenicol and 1 % glucose (2 x TY-CG). After helper phage infection (VCSM13) at an OD600 of 0.5 (30 min at 37°C without shaking; 30 min at 37°C shaking at 250 rpm), cells were spun down (4120 g; 5 min; 4°C), resuspended in 2 x TY / 34 µg/ml chloramphenicol / 50 µg/ml kanamycin and grown overnight at 22°C. Phages were PEG-precipitated from the supernatant, resuspended in PBS / 20 % glycerol and stored at -80°C. Phage amplification between two panning rounds was conducted as follows: mid-log phase TG1 cells were infected with eluted phages and plated onto LB-agar supplemented with 1 % of glucose and 34 µg/ml of chloramphenicol (LB-CG). After overnight incubation at 30°C, colonies were scraped off, adjusted to an OD600 of 0.5 and helper phage added as described above.

B. Pannings with HuCAL® GOLD

For the selections HuCAL® GOLD antibody-phages were divided into three pools corresponding to different VH master genes (pool 1: VH1/5λκ, pool 2: VH3 λκ, pool 3: VH2/4 λκ). These pools were individually subjected to 3 rounds of a whole cell panning on CD38-expressing CHO cells followed by pH-elution and a post-adsorption step on CD38-negative CHO-cells for depletion of irrelevant antibody-phages. Finally, the remaining antibody phages were used to infect E.

infect E. coli TG1 cells. After centrifugation the bacterial pellet was resuspended in 2 x TY medium, plated on agar plates and incubated overnight at 30°C. The selected clones were then scraped from the plates, rescued and amplified. The second and the third round of selections was performed as the initial one.

- 5 The Fab encoding inserts of the selected HuCAL® GOLD phages were subcloned into the expression vector pMORPHx9_Fab_FS (Rauchenberger et al., 2003) to facilitate rapid expression of soluble Fab. The DNA of the selected clones was digested with XbaI and EcoRI thereby cutting out the Fab encoding insert (ompA-VLCL and phoA-Fd), and cloned into the XbaI / EcoRI cut vector
10 pMORPHx9_Fab_FS. Fab expressed in this vector carry two C-terminal tags (FLAG™ and Strep-tag® II) for detection and purification.

EXAMPLE 2: Biological assays

- Antibody dependent cellular cytotoxicity (ADCC) and complement-dependent
15 cytotoxicity was measured according to a published protocol based on flow-cytometry analysis (Naundorf et al., 2002) as follows:

ADCC:

- For ADCC measurements, target cells (T) were adjusted to 2.0E+05 cells/ml and
20 labeled with 100 ng/ml Calcein AM (Molecular Probes, C-3099) in RPMI1640 medium (Pan) for 2 minutes at room-temperature. Residual calcein was removed by 3 washing steps in RPMI1640 medium. In parallel PBMC were prepared as source for (natural killer) effector cells (E), adjusted to 1.0E+07 and mixed with the labeled target cells to yield a final E:T-ratio of 50:1 or less, depending on the
25 assay conditions. Cells were washed once and the cell-mix resuspended in 200 µl RPMI8226 medium containing the respective antibody at different dilutions. The

plate was incubated for 4 hrs under standardized conditions at 37°C and 5% CO₂ in a humidified incubator. Prior to FACS analysis cells were labelled with propidium-iodide (PI) and analyzed by flow-cytometry (Becton-Dickinson). Between 50,000 and 150,000 events were counted for each assay.

- 5 The following equation gave rise to the killing activity [in %]:

$$\frac{ED^A}{EL^A + ED^A} \times 100$$

10

with ED^A = events dead cells (calcein + PI stained cells), and
 EL^A = events living cells (calcein stained cells)

CDC:

- 15 For CDC measurements, 5.0E+04 CD38 CHO-transfectants were added to a microtiter well plate (Nunc) together with a 1:4 dilution of human serum (Sigma, #S-1764) and the respective antibody. All reagents and cells were diluted in RPMI1640 medium (Pan) supplemented with 10% FCS. The reaction-mix was incubated for 2 hrs under standardized conditions at 37°C and 5% CO₂ in a
20 humidified incubator. As negative controls served either heat-inactivated complement or CD38-transfectants without antibody. Cells were labelled with PI and subjected to FACS-analysis.

- In total 5000 events were counted and the number of dead cells at different antibody concentrations used for the determination of EC₅₀ values. The following
25 equation gave rise to the killing activity [in %]:

$$\frac{ED^C}{EL^C + ED^C} \times 100$$

- 30 with ED^C = events dead cells (PI stained cells), and
 EL^C = events living cells (unstained)

Cytotoxicity values from a total of 12 different antibody-dilutions (1:2n) in triplicates were used in ADCC and CDC for for each antibody in order obtain EC-values with a standard analysis software (PRISM[®], Graph Pad Software).

5 **EXAMPLE 3: Generation of stable CD38-transfectants and CD38 Fc-fusion proteins**

In order to generate CD38 protein for panning and screening two different expression-systems had to be established. The first strategy included the generation of CD38-Fc-fusion protein which was purified from supernatants after transient transfection of HEK293 cells. The second strategy involved the
10 generation of a stable CHO-cell-line for high CD38-surface expression to be used for selection of antibody-phages via whole cell panning.

As an initial step Jurkat cells (DSMZ ACC282) were used for the generation of cDNA (Invitrogen) followed by amplification of the entire CD38-coding sequence using primers complementary to the first 7 and the last 9 codons of CD38,
15 respectively (primer MT001 & 002rev; Table 4). Sequence analysis of the CD38-insert confirmed the published aa-sequence by Jackson et al. (1990) except for position 49 which revealed a glutamine instead of a tyrosine as described by Nata et al. (1997). For introduction of restriction-endonuclease sites and cloning into different derivatives of expression vector pcDNA3.1 (Stratagene), the purified
20 PCR-product served as a template for the re-amplification of the entire gene (primers MTE006 & 007rev, Table 4) or a part (primers MTE004 & 009rev, Table 4) of it. In the latter case a fragment encoding for the extracellular domain (aa 45 to 300) was amplified and cloned in frame between a human Vkappa leader sequence and a human Fc-gamma 1 tag sequence. This vector served as
25 expression vector for the generation of soluble CD38 fusion-protein. Another pcDNA3.1-derivative without leader-sequence was used for insertion of the CD38 full-length gene. In this case a stop codon in front of the Fc-coding region and the missing leader-sequence gave rise to CD38-surface expression. HEK293 cells were transiently transfected with the Fc-fusion protein vector for generation
30 of soluble CD38 Fc-fusion protein and, in case of the full-length derivative, CHO-cells were transfected for the generation of a stable CD38-expressing cell line.

Table 4:

Primer #	Sequence (5'→3')
MTE001	ATG GCC AAC TGC GAG TTC AGC (SEQ ID NO: 25)
MTE002rev	TCA GAT CTC AGA TGT GCA AGA TGA ATC (SEQ ID NO: 26)
MTE004	TT GGT ACC AGG TGG CGC CAG CAG TG (SEQ ID NO: 27)
MTE006	TT GGT ACC ATG GCC AAC TGC GAG (SEQ ID NO: 28)
MTE007rev	CCG ATA TCA* GAT CTC AGA TGT GCA AGA TG (SEQ ID NO: 29)
MTE009rev	CCG ATA TC GAT CTC AGA TGT GCA AGA TG (SEQ ID NO: 30)

* leading to a stop codon (TGA) in the sense orientation.

5 EXAMPLE 4: Cloning, expression and purification of HuCAL[®] IgG1:

In order to express full length IgG, variable domain fragments of heavy (VH) and light chains (VL) were subcloned from Fab expression vectors into appropriate pMorph_hIg vectors (see Figures 8 to 10). Restriction endonuclease pairs *BlnI/MfeI* (insert-preparation) and *BlnI/EcoRI* (vector-preparation) were used for subcloning of the VH domain fragment into pMorph_hIgG1. Enzyme-pairs *EcoRV/HpaI* (lambda-insert) and *EcoRV/BsiWI* (kappa-insert) were used for subcloning of the VL domain fragment into the respective pMorph_hIgκ_1 or pMorph_h_Igλ_1 vectors. Resulting IgG constructs were expressed in HEK293 cells (ATCC CRL-1573) by transient transfection using standard calcium phosphate-DNA-coprecipitation technique.

IgGs were purified from cell culture supernatants by affinity chromatography via Protein A Sepharose column. Further down stream processing included a buffer exchange by gel filtration and sterile filtration of purified IgG. Quality control revealed a purity of >90 % by reducing SDS-PAGE and >90 % monomeric IgG as determined by analytical size exclusion chromatography. The endotoxin content of the material was determined by a kinetic LAL based assay (Cambrex European Endotoxin Testing Service, Belgium).

EXAMPLE 5: Generation and production of chimeric OKT10 (chOKT10; SEQ ID NOS: 23 and 24)

For the construction of chOKT10 the mouse VH and VL regions were amplified by PCR using cDNA prepared from the murine OKT10 hybridoma cell line (ECACC #87021903). A set of primers were used as published (Dattamajumdar et al., 1996; Zhou et al., 1994). PCR products were used for Topo-cloning (Invitrogen; pCRII-vector) and single colonies subjected to sequence analysis (M13 reverse primer) which revealed two different kappa light chain sequences and one heavy chain sequence. According to sequence alignments (EMBL-nucleotide sequence database) and literature (Krebber et al, 1997) one of the kappa-sequence belongs to the intrinsic repertoire of the tumor cell fusion partner X63Ag8.653 and hence does not belong to OKT10 antibody. Therefore, only the new kappa sequence and the single VH-fragment was used for further cloning. Both fragments were reamplified for the addition of restriction endonuclease sites followed by cloning into the respective pMorph IgG1-expression vectors. The sequences for the heavy chain (SEQ ID NO: 23) and light chain (SEQ ID NO: 24) are given in Fig. 6. HEK293 cells were transfected transiently and the supernatant analyzed in FACS for the chimeric OKT10 antibody binding to the CD38 over-expressing Raji cell line (ATCC).

EXAMPLE 6: Epitope Mapping**1. Materials and Methods:****Antibodies:**

The following anti-CD38 IgGs were sent for epitope mappings:

MOR#	Lot #	Format	Conc. [mg/ml]/Vol.[μl]
MOR03077	2CHE106_030602	human IgG1	0.44/1500
MOR03079	2APO31	human IgG1	0.38/500
MOR03080	030116_4CUE16	human IgG1	2.28/200
MOR03100	030612_6SBA6	human IgG1	0.39/500
chim. OKT10*	030603_2CHE111	human IgG1	0.83/500

* chimeric OKT10 consisting of human Fc and mouse variable regions.

CD38-Sequence:

The amino acid (aa) sequence (position 44 – 300) is based on human CD38 taken from the published sequence under SWISS-PROT primary accession number P28907. At position 49 the aa Q (instead of T) has been used for the peptide-design.

PepSpot-Analysis:

The antigen peptides were synthesized on a cellulose membrane in a stepwise manner resulting in a defined arrangement (peptide array) and are covalently bound to the cellulose membrane. Binding assays were performed directly on the peptide array.

In general an antigen peptide array is incubated with blocking buffer for several hours to reduce non-specific binding of the antibodies. The incubation with the primary (antigen peptide-binding) antibody in blocking buffer occurs followed by the incubation with the peroxidase (POD)-labelled secondary antibody, which binds selectively the primary antibody. A short T(Tween)-TBS-buffer washing directly after the incubation of the antigen peptide array with the secondary antibody followed by the first chemiluminescence experiment is made to get a first overview which antigen peptides do bind the primary antibody. Several buffer washing steps follow (T-TBS- and TBS-buffer) to reduce false positive binding (unspecific antibody binding to the cellulose membrane itself). After these washing steps the final chemiluminescence analysis is performed. The data were analysed with an imaging system showing the signal intensity (Boehringer Light units, BLU) as single measurements for each peptide. In order to evaluate non-specific binding of the secondary antibodies (anti-human IgG), these antibodies were incubated with the peptide array in the absence of primary antibodies as the first step. If the primary antibody does not show any binding to the peptides it can be directly labelled with POD, which increases the sensitivity of the system (as performed for MOR3077). In this case a conventional coupling chemistry *via* free amino-groups is performed.

The antigen was scanned with 13-mer peptides (11 amino acids overlap). This resulted in arrays of 123 peptides. Binding assays were performed directly on the array. The peptide-bound antibodies MOR03077, MOR03079, MOR03080, MOR03100 and chimeric OKT10 were detected using a peroxidase-labelled

secondary antibody (peroxidase conjugate-goat anti-human IgG, gamma chain specific, affinity isolated antibody; Sigma-Aldrich, A6029). The mappings were performed with a chemiluminescence substrate in combination with an imaging system. Additionally, a direct POD-labelling of MOR03077 was performed in
5 order to increase the sensitivity of the system.

2. Summary and Conclusions:

All five antibodies showed different profiles in the PepSpot analysis. A schematic summary is given in Fig. 7, which illustrates the different aa sequences of CD38
10 being recognized. The epitope for MOR03079 and chimeric OKT10 can clearly be considered as linear. The epitope for MOR03079 can be postulated within aa 192 – 206 (VSRRFAEAACDVVHV) of CD38 whereas for chimeric OKT10 a sequence between aa 284 and 298 (FLQCVKNPEDSSCTS) is recognized predominantly. The latter results confirm the published data for the parental
15 murine OKT10 (Hoshino *et al.*, 1997), which postulate its epitope between aa 280-298. Yet, for a more precise epitope definition and determination of key amino acids (main antigen-antibody interaction sites) a shortening of peptides VSRRFAEAACDVVHV and FLQCVKNPEDSSCTS and an alanine-scan of both should be envisaged.

20 The epitopes for MOR03080 and MOR03100 can be clearly considered as discontinuous since several peptides covering different sites of the protein sites were recognized. Those peptides comprise aa 82-94 and aa 158-170 for MOR03080 and aa 82-94, 142-154, 158-170, 188-200 and 280-296 for MOR03100. However, some overlaps between both epitopes can be postulated
25 since two different sites residing within aa positions 82-94 (CQSVWDAFKGAFI; peptide #20) and 158-170 (TWCGEFNTSKINY; peptide #58) are recognized by both antibodies.

The epitope for MOR03077 can be considered as clearly different from the latter two and can be described as multisegmented discontinuous epitope. The
30 epitope includes aa 44-66, 110-122, 148-164, 186-200 and 202-224.

References:

- Ausiello C.M., Urbani F., Lande R., la Sala A., Di Carlo B., Baj G., Surico N., Hilgers J., Deaglio S., Funaro A., Malavasi F. (2000) Functional topography of discrete domains of human CD38. *Tissue Antigens*. 2000 Dec;56(6):539-47.
- 5 Chamow, S.M., Zhang, D.Z., Tan, X.Y., Mathre, S.M., Marsters, S.A., Peers, D.H., Byrn, R.A., Ashknazi, A., Junghans, R.P (1994). humanized, bispecific immunoadhesin-antibody that retargets CD3+ effectors to kill HIV-1-infected cells. *J Immunol*. 1994 Nov 1;153(9):4268-80
- 10 Dattamajumdar, A.K., Jacobsen, D.P., Hood, L.E., Osman, G.E. (1996). Rapid cloning of rearranged muse immunoglobulin variable genes. *Immunogenetics* 43, 141-151
- 15 Funaro, A., Spagnoli, G.C., Ausiello, C.M., Alessio, M., Roggero, S., Delia, D., Zaccolo, M., and Malavasi, F. (1990) Involvement of the multilineage CD38 molecule in a unique pathway of cell activation and proliferation. *J. Immunol*. 145, 2390-2396.
- 20 Hoshino S., Kukimoto I., Kontani K., Inoue S., Kanda Y., Malavasi F., Katada T. (1997) Mapping of the catalytic and epitopic sites of human CD38/NAD+ glycohydrolase to a functional domain in the carboxyl terminus. *J Immunol*. 158(2):741-7.
- 25 Jackson D.G., Bell J.I. (1990) Isolation of a cDNA encoding the human CD38 (T10) molecule, a cell surface glycoprotein with an unusual discontinuous pattern of expression during lymphocyte differentiation. *J Immunol*. 144(7):2811-5.
- 30 Knappik, A., Ge, L., Honegger, A., Pack, P., Fischer, M., Wellnhofer, G., Hoess, A., Wolle, J., Pluckthun, A., and Virnekas, B. (2000). Fully synthetic human combinatorial antibody libraries (HuCAL) based on modular consensus frameworks and CDRs randomized with trinucleotides. *J Mol Biol* 296, 57-86.
- 35 Konopleva M., Estrov Z., Zhao S., Andreeff M., Mehta K. (1998) Ligation of cell surface CD38 protein with agonistic monoclonal antibody induces a cell growth signal in myeloid leukemia cells. *J Immunol*. 161(9):4702-8.
- 40 Krebber, A., Bornhauser, S., Burmester, J., Honegger, A., Willuda, J., Bossard, H.R., Pluckthun, A. (1997). Reliable cloning of functional antibody variable domains from hybridomas and spleen cell repertoires employing a reengineered phage display system. *J. Imm. Meth.* 201, 35-55.
- 45 Krebs, B., Rauchenberger, R., Reiffert, S., Rothe, C., Tesar, M., Thomassen, E., Cao, M., Dreier, T., Fischer, D., Hoss, A., Inge, L., Knappik, A., Marget, M., Pack, P., Meng, X.Q., Schier, R., Sohlmann, P., Winter, J., Wolle, J., and Kretzschmar, T. (2001). High-throughput generation and engineering of recombinant human antibodies. *J Immunol Methods* 254, 67-84.
- 50 Löhning, C. (2001). Novel methods for displaying (poly)peptides/proteins on bacteriophage particles via disulfide bonds. WO 01/05950.

Maloney D.G., Donovan K., Hamblin T.J. (1999) Antibody therapy for treatment of multiple myeloma. *Semin Hematol.* 36(1 Suppl 3):30-3

- 5 Nata K., Takamura T., Karasawa T., Kumagai T., Hashioka W., Tohgo A., Yonekura H., Takasawa S., Nakamura S., Okamoto H. (1997) Human gene encoding CD38 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase): organization, nucleotide sequence and alternative splicing. *Gene* 186(2):285-92.
- 10 Naundorf, S., Preithner, S., Mayer, P., Lippold, S., Wolf, A., Hanakam, F., Fichtner, I., Kufer, P., Raum, T., Riethmüller, G., Baeuerle, P.A., Dreier, T. (2002). *Int. J. Cancer* 100, 101-110.
- 15 Rauchenberger R., Borges E., Thomassen-Wolf E., Rom E., Adar R., Yaniv Y., Malka M., Chumakov I., Kotzer S., Resnitzky D., Knappik A., Reiffert S., Prassler J., Jury K., Waldherr D., Bauer S., Kretzschmar T., Yayon A., Rothe C. (2003) Human combinatorial Fab library yielding specific and functional antibodies against the human fibroblast growth factor receptor 3. *J Biol Chem.* 278(40):38194-205.
- 20 Zhou, H., Fisher, R.J., Papas, T.S. (1994). Optimization of primer sequences for mouse scFv repertoire display library construction. *Nucleic Acids Res.* 22: 888-889.

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CLAIMS

1. An isolated human antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of CD38 (SEQ ID NO: 22), wherein said human antibody or functional fragment thereof is able to mediate killing of a CD38+ target cell by ADCC with an at least five-fold better efficacy than chimeric OKT10 (SEQ ID NOS: 23 and 24) under the same or substantially the same conditions when a human PBMC cell is employed as an effector cell, wherein said CD38+ target cell is selected from the group consisting of LP-1 (DSMZ: ACC41) and RPMI-8226 (ATCC: CCL-155), and wherein the ratio of target cells to effector cells is between about 30:1 and about 50:1.
2. An antigen-binding region of a human antibody or functional fragment thereof according to claim 1.
3. An antigen-binding region according to claim 2, which comprises an H-CDR3 region depicted in SEQ ID NO: 5, 6, 7, or 8.
4. An antigen-binding region according to claim 3, further comprising an H-CDR2 region depicted in SEQ ID NO: 5, 6, 7, or 8.
5. An antigen-binding region according to claim 4, further comprising an H-CDR1 region depicted in SEQ ID NO: 5, 6, 7, or 8.
6. An antigen-binding region according to claim 5, which comprises a variable heavy chain depicted in SEQ ID NO: 5, 6, 7, or 8.
7. An antigen-binding region according to claim 2, which comprises an L-CDR3 region depicted in SEQ ID NO: 13, 14, 15, or 16.
8. An antigen-binding region according to claim 7, further comprising an L-CDR1 region depicted in SEQ ID NO: 13, 14, 15, or 16.

9. An antigen-binding region according to claim 8, further comprising an L-CDR2 region depicted in SEQ ID NO: 13, 14, 15, or 16.
10. An antigen-binding region according to claim 9, which comprises a variable light chain depicted in SEQ ID NO: 13, 14, 15, or 16.
11. An antigen-binding region according to claim 2, which comprises a heavy chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 5, 6, 7, or 8; (ii) a sequence having at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 6, 7, or 8; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 6, 7, or 8.
12. An antigen-binding region according to claim 2, which comprises a light chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 13, 14, 15, or 16; (ii) a sequence having at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 14, 15, or 16; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 14, 15, or 16.
13. An antibody according to claims 1, which is an IgG.
14. An antibody according to claim 13, which is an IgG1.
15. An isolated human antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of CD38 (SEQ ID NO: 22), wherein said human antibody or functional fragment thereof is able to mediate killing of a CD38-transfected CHO cell by CDC with an at least two-fold better efficacy than chimeric OKT10 (SEQ ID NOS: 23 and 24) under the same or substantially the same conditions.

16. An antigen-binding region of a human antibody or functional fragment thereof according to claim 15.

17. An antigen-binding region according to claim 16, which comprises an H-CDR3 region depicted in SEQ ID NO: 5, 6, or 7.

18. An antigen-binding region according to claim 17, further comprising an H-CDR2 region depicted in SEQ ID NO: 5, 6, or 7.

19. An antigen-binding region according to claim 18, further comprising an H-CDR1 region depicted in SEQ ID NO: 5, 6, or 7.

20. An antigen-binding region according to claim 19, which comprises a variable heavy chain depicted in SEQ ID NO: 5, 6, or 7.

21. An antigen-binding region according to claim 16, which comprises an L-CDR3 region depicted in SEQ ID NO: 13, 14, or 15.

22. An antigen-binding region according to claim 21, further comprising an L-CDR1 region depicted in SEQ ID NO: 13, 14, or 15.

23. An antigen-binding region according to claim 22, further comprising an L-CDR2 region depicted in SEQ ID NO: 13, 14, or 15.

24. An antigen-binding region according to claim 23, which comprises a variable light chain depicted in SEQ ID NO: 13, 14, or 15.

25. An antigen-binding region according to claim 16, which comprises a heavy chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 5, 6, or 7; (ii) a sequence having at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 6, or 7; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 6, or 7.

26. An antigen-binding region according to claim 16, which comprises a light chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 13, 14, or 15; (ii) a sequence having at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 14, or 15; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 14, or 15.

27. An antibody according to claims 15, which is an IgG.

28. An antibody according to claim 27, which is an IgG1.

29. An isolated human antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of CD38, wherein the epitope comprises one or more amino acid residues of amino acid residues 1 to 215 of CD38 (SEQ ID NO: 22).

30. An isolated human antibody or functional fragment thereof of claim 29, wherein the epitope comprises one or more amino acid residues comprised in one or more of the amino acid stretches taken from the list of amino acid stretches 44-66, 82-94, 142-154, 148-164, 158-170, and 192-206 of CD38.

31. An antibody or functional fragment thereof according to claim 29, wherein said epitope is a linear epitope.

32. An antibody or functional fragment thereof according to claim 31, wherein said antigen-binding region comprises an H-CDR3 region depicted in SEQ ID NO: 6.

33. An antibody or functional fragment thereof according to claim 32, wherein said antigen-binding region further comprises an H-CDR2 region depicted in SEQ ID NO: 6.

34. An antibody or functional fragment thereof according to claim 33, wherein said antigen-binding region further comprises an H-CDR1 region depicted in SEQ ID NO: 6.

35. An antibody or functional fragment thereof according to claim 31, which comprises a variable heavy chain depicted in SEQ ID NO: 6.

36. An antibody or functional fragment thereof according to claim 31, wherein said antigen-binding region comprises an L-CDR3 region depicted in SEQ ID NO: 14.

37. An antibody or functional fragment thereof according to claim 36, wherein said antigen-binding region further comprises an L-CDR1 region depicted in SEQ ID NO: 14.

38. An antibody or functional fragment thereof according to claim 37, wherein said antigen-binding region further comprises an L-CDR2 region depicted in SEQ ID NO: 14.

39. An antibody or functional fragment thereof according to claim 31, which comprises a variable light chain depicted in SEQ ID NO: 14.

40. An antibody or functional fragment thereof according to claim 31, which comprises a heavy chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 14; (ii) a sequence having at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 14; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 14.

41. An antibody or functional fragment thereof according to claim 31, which comprises a light chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 14; (ii) a sequence having at least 60 percent

sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 14; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 14.

42. A functional fragment according to claim 31, which is a Fab or scFv antibody fragment.

43. An antibody according to claims 31, which is an IgG.

44. An antibody according to claim 43, which is an IgG1.

45. An antibody or functional fragment thereof according to claim 11, wherein said epitope is a conformational epitope.

46. An antibody or functional fragment thereof according to claim 45, wherein said antigen-binding region comprises an H-CDR3 region depicted in SEQ ID NO: 5, 7, or 8.

47. An antibody or functional fragment thereof according to claim 46, wherein said antigen-binding region further comprises an H-CDR2 region depicted in SEQ ID NO: 5, 7, or 8.

48. An antibody or functional fragment thereof according to claim 47, wherein said antigen-binding region further comprises an H-CDR1 region depicted in SEQ ID NO: 5, 7, or 8.

49. An antibody or functional fragment thereof according to claim 45, which comprises a variable heavy chain depicted in SEQ ID NO: 5, 7, or 8.

50. An antibody or functional fragment thereof according to claim 45, wherein said antigen-binding region comprises an L-CDR3 region depicted in SEQ ID NO: 13, 15, or 16.

51. An antibody or functional fragment thereof according to claim 50, wherein said antigen-binding region further comprises an L-CDR1 region depicted in SEQ ID NO: 13, 15, or 16.

52. An antibody or functional fragment thereof according to claim 51, wherein said antigen-binding region further comprises an L-CDR2 region depicted in SEQ ID NO: 13, 15, or 16.

53. An antibody or functional fragment thereof according to claim 45, which comprises a variable light chain depicted in SEQ ID NO: 13, 15, or 16.

54. An antibody or functional fragment thereof according to claim 45, which comprises a heavy chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 5, 7, or 8; (ii) a sequence having at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 7, or 8; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 7, or 8.

55. An antibody or functional fragment thereof according to claim 45, which comprises a light chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 13, 15, or 16; (ii) a sequence having at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 15, or 16; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 15, or 16.

56. A functional fragment according to claim 45, which is a Fab or scFv antibody fragment.

57. An antibody according to claims 45, which is an IgG.

58. An antibody according to claim 57, which is an IgG1.

59. A variable heavy chain of an isolated antibody or functional fragment thereof that is encoded by (i) a nucleic acid sequence comprising SEQ ID NO: 1, 2, 3, or 4, or (ii) a nucleic acid sequences that hybridizes under high stringency conditions to the complimentary strand of SEQ ID NO: 1, 2, 3, or 4, wherein said antibody or functional fragment thereof is specific for an epitope of CD38.

60. A variable light chain of an isolated antibody or functional fragment thereof that is encoded by (i) a nucleic acid sequence comprising SEQ ID NO: 9, 10, 11, or 12, or (ii) a nucleic acid sequences that hybridizes under high stringency conditions to the complimentary strand of SEQ ID NO: 9, 10, 11, or 12, wherein said antibody or functional fragment thereof is specific for an epitope of CD38.

61. An isolated nucleic acid sequence that encodes an antigen-binding region of a human antibody or functional fragment thereof that is specific for an epitope of CD38.

62. A nucleic acid sequence encoding a variable heavy chain of an isolated antibody or functional fragment thereof, which comprises (i) a sequence selected from the group consisting of SEQ ID NOS: 1, 2, 3 and 4 or (ii) a nucleic acid sequence that hybridizes under high stringency conditions to the complimentary strand of SEQ ID NO: 1, 2, 3 or 4, wherein said antibody or functional fragment thereof is specific for an epitope of CD38.

63. A nucleic acid sequence encoding a variable light chain of an isolated antibody or functional fragment thereof, which comprises (i) a sequence selected from the group consisting of SEQ ID NOS: 9, 10, 11 and 12 or (ii) a nucleic acid sequence that hybridizes under high stringency conditions to the

complimentary strand of SEQ ID NO: 9, 10, 11 or 12, wherein said antibody or functional fragment thereof is specific for an epitope of CD38.

64. A vector comprising a nucleic acid sequence according to any one of claims 61-63.

65. An isolated cell comprising a vector according to claim 64.

66. An isolated cell according to claim 65, wherein said cell is bacterial.

67. An isolated cell according to claim 65, wherein said cell is mammalian.

68. A pharmaceutical composition comprising an antibody or functional fragment according to any one of claims 1, 15 and 29 and a pharmaceutically acceptable carrier or excipient therefor.

69. A method for treating a disorder or condition associated with the undesired presence of CD38+ cells, comprising administering to a subject in need thereof an effective amount of the pharmaceutical composition according to claim 68.

70. A method according to claim 69, wherein said disorder or condition is a haematological disease.

71. A method according to claim 70 taken from the list of multiple myeloma, chronic lymphocytic leukemia, chronic myelogenous leukemia, acute myelogenous leukemia, and acute lymphocytic leukemia.

72. A method according to claim 69, wherein said disorder or condition is an inflammatory disease

73. A method according to claim 72 taken from the list of rheumatoid arthritis and systemic lupus erythematosus.

74. A method for targeting CD38+ cells in a subject or a cell sample, comprising the step of allowing said CD38+ cells to be contacted with an antibody or functional fragment thereof according to any one of claims 1, 15 and 29.

ABSTRACT

The present invention provides recombinant antigen-binding regions and antibodies and functional fragments containing such antigen-binding regions that are specific for CD38, which plays an integral role in various disorders or conditions. These antibodies, accordingly, can be used to treat, for example, hematological malignancies such as multiple myeloma. Antibodies of the invention also can be used in the diagnostics field, as well as for investigating the role of CD38 in the progression of disorders associated with malignancies. The invention also provides nucleic acid sequences encoding the foregoing antibodies, vectors containing the same, pharmaceutical compositions and kits with instructions for use.

Figure 1a

Variable Heavy Chain DNA

3077_VH1B (SEQ ID NO: 1):

(1) CAGGTGCAAT TGGTTCAGAG CGGCGCGGAA GTGAAAAAAC CGGGCGCGAG
(51) CGTGAAAGTG AGCTGCAAAG CCTCCGGATA TACCTTTACT TCTTATTCTA
(101) TTAATTGGGT CCGCCAAGCC CCTGGGCAGG GTCTCGAGTG GATGGGCTAT
(151) ATCGATCCGA ATCGTGGCAA TACGAATTAC GCGCAGAAAGT TTCAGGGCCG
(201) GGTGACCATG ACCCGTGATA CCAGCATTAG CACCGCGTAT ATGGAAGTGA
(251) GCAGCCTGCG TAGCGAAGAT ACGGCCGTGT ATTATTGCGC GCGTGAGTAT
(301) ATTTATTTTA TTCATGGTAT GCTTGATTTT TGGGGCCAAG GCACCCTGGT
(351) GACGGTTAGC TCA

3079_VH3 (SEQ ID NO: 2):

(1) CAGGTGCAAT TGGTGGAAG CGGCGGCGGC CTGGTGCAAC CGGGCGGCAG
(51) CCTGCGTCTG AGCTGCGCGG CCTCCGGATT TACCTTTTCT AATTATGGTA
(101) TGCATTGGGT GCGCCAAGCC CCTGGGAAGG GTCTCGAGTG GGTGAGCAAT
(151) ATCCGTCTG ATGGTAGCTG GACCTATTAT GCGGATAGCG TGAAAGGCCG
(201) TTTTACCATT TCACGTGATA ATTCGAAAAA CACCCTGTAT CTGCAAATGA
(251) ACAGCCTGCG TGCGGAAGAT ACGGCCGTGT ATTATTGCGC GCGTCGTTAT
(301) TGGTCTAAGT CTCATGCTTC TGTTACTGAT TATTGGGGCC AAGGCACCCT
(351) GGTGACGGTT AGCTCA

3080_VH3 (SEQ ID NO: 3):

(1) CAGGTGCAAT TGGTGGAAG CGGCGGCGGC CTGGTGCAAC CGGGCGGCAG
(51) CCTGCGTCTG AGCTGCGCGG CCTCCGGATT TACCTTTTCT TCTTATGGTA
(101) TGCATTGGGT GCGCCAAGCC CCTGGGAAGG GTCTCGAGTG GGTGAGCAAT
(151) ATCTATTCTG ATGGTAGCAA TACCTTTTAT GCGGATAGCG TGAAAGGCCG
(201) TTTTACCATT TCACGTGATA ATTCGAAAAA CACCCTGTAT CTGCAAATGA
(251) ACAGCCTGCG TGCGGAAGAT ACGGCCGTGT ATTATTGCGC GCGTAATATG
(301) TATCGTTGGC CTTTTCATTA TTTTTTGGAT TATTGGGGCC AAGGCACCCT
(351) GGTGACGGTT AGCTCA

3100_VH 3 (SEQ ID NO: 4):

(1) CAGGTGCAAT TGGTGGAAG CGGCGGCGGC CTGGTGCAAC CGGGCGGCAG
(51) CCTGCGTCTG AGCTGCGCGG CCTCCGGATT TACCTTTTCT TCTAATGGTA
(101) TGTCTTGGGT GCGCCAAGCC CCTGGGAAGG GTCTCGAGTG GGTGAGCAAT
(151) ATCTCTTATC TTTCTAGCTC TACCTATTAT GCGGATAGCG TGAAAGGCCG
(201) TTTTACCATT TCACGTGATA ATTCGAAAAA CACCCTGTAT CTGCAAATGA
(251) ACAGCCTGCG TGCGGAAGAT ACGGCCGTGT ATTATTGCGC GCGTTTTTAT
(301) GGTATTATTTA ATTATGCTGA TGTTTGGGGC CAAGGCACCC TGGTGACGGT
(351) TAGCTCA

3077_1_VH1B (SEQ ID NO: 31):

(1) CAGGTGCAAT TAGTCCAAAG TGGTGCGGAA GTGAAAAAAC CGGGCGCGAG
(51) CGTGAAAGTG AGCTGCAAAG CCTCCGGATA TACCTTTACT TCTTATTCTA
(101) TTAATTGGGT CCGCCAAGCC CCTGGGCAGG GTCTCGAGTG GATGGGCTAT
(151) ATCGATCCGA ATCGTGGCAA TACGAATTAC GCGCAGAAAGT TTCAGGGCCG
(201) GGTGACCATG ACCCGTGATA CCAGCATTAG CACCGCGTAT ATGGAAGTGA
(251) GCAGCCTGCG TAGCGAAGAT ACGGCCGTGT ATTATTGCGC GCGTGAGTAT

(301) ATTTATTTTA TTCATGGTAT GCTTGATTTT TGGGGCCAAG GCACCCTGGT
(351) GACGGTTAGC TCA

Figure 1b

Variable Heavy Chain Peptide

(CDR Regions in Bold)

3077_VH1B (SEQ ID NO: 5):

(1) QVQLVQSGAE VKKPGASVKV SCKASGYTFT **SY**SINWVRQA PGQGLEWMGY
(51) IDPNRGNTNY **AQKFQ**GRVTM TRDTSISTAY MELSSLRSED TAVYYCAREY
(101) **IYFIHGMLDF** WGQGTLVTVS S

3079_VH3 (SEQ ID NO: 6):

(1) QVQLVESGGG LVQPGGSLRL SCAASGFTFS **NYGM**HWRQA PGKGLEWVSN
(51) **IRSDGS**WTYY **ADSVK**GRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARRY
(101) **WSKSHAS**VD YWGQGTLLTV SS

3080_VH3 (SEQ ID NO: 7):

(1) QVQLVESGGG LVQPGGSLRL SCAASGFTFS **SYGM**HWRQA PGKGLEWVSN
(51) **IYSDGS**NTFY **ADSVK**GRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARNM
(101) **YRWPFHYFFD** YWGQGTLLTV SS

3100_VH 3 (SEQ ID NO: 8):

(1) QVQLVESGGG LVQPGGSLRL SCAASGFTFS **SNGM**SWVRQA PGKGLEWVSN
(51) **ISYLSS**STYY **ADSVK**GRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARFY
(101) **GYFNYAD**VWG QGTLLTVSS

Figure 2a

Variable Light Chain DNA

3077_Vk kappa 2 (SEQ ID NO: 9):

```
(1)  GATATCGTGA TGACCCAGAG CCCACTGAGC CTGCCAGTGA CTCCGGGCGA
(51) GCCTGCGAGC ATTAGCTGCA GAAGCAGCCA AAGCCTGCTT TTTATTGATG
(101) GCAATAATTA TCTGAATTGG TACCTTCAAA AACCAGGTCA AAGCCCGCAG
(151) CTATTAATTT ATCTTGGTTC TAATCGTGCC AGTGGGGTCC CGGATCGTTT
(201) TAGCGGCTCT GGATCCGGCA CCGATTTTAC CCTGAAAATT AGCCGTGTGG
(251) AAGCTGAAGA CGTGGGCGTG TATTATTGCC AGCAGTATTC TTCTAAGTCT
(301) GCTACCTTTG GCCAGGGTAC GAAAGTTGAA ATTAAACGTA CG
```

3079_Vk kappa 1 (SEQ ID NO: 10):

```
(1)  GATATCCAGA TGACCCAGAG CCCGTCTAGC CTGAGCGCGA GCGTGGGTGA
(51) TCGTGTGACC ATTACCTGCA GAGCGAGCCA GGATATTTCT GCTTTTCTGA
(101) ATTGGTACCA GCAGAAACCA GGTAAGCAC CGAAACTATT AATTTATAAG
(151) GTTTCTAATT TGCAAAGCGG GGTCCCGTCC CGTTTtagCG GCTCTGGATC
(201) CGGCACTGAT TTTACCCCTGA CCATTAGCAG CCTGCAACCT GAAGACTTTG
(251) CGACTTATTA TTGCCAGCAG GCTTATTCTG GTTCTATTAC CTTTGGCCAG
(301) GGTACGAAAG TTGAAATTAA ACGTACG
```

3080_VI lambda 3 (SEQ ID NO: 11):

```
(1)  GATATCGAAC TGACCCAGCC GCCTTCAGTG AGCGTTGCAC CAGGTCAGAC
(51) CGCGCGTATC TCGTGTAGCG GCGATAATAT TGGTAATAAG TATGTTTCTT
(101) GGTACCAGCA GAAACCCGGG CAGGCGCCAG TTGTTGTGAT TTATGGTGAT
(151) AATAATCGTC CCTCAGGCAT CCCGGAACGC TTTAGCGGAT CCAACAGCGG
(201) CAACACCGCG ACCCTGACCA TTAGCGGCAC TCAGGCGGAA GACGAAGCGG
(251) ATTATTATTG CTCTTCTTAT GATTCTTCTT ATTTTGTGTT TGGCGGCGGC
(301) ACGAAGTTAA CCGTTCTTGG CCAG
```

3100_VI lambda 3 (SEQ ID NO: 12):

```
(1)  GATATCGAAC TGACCCAGCC GCCTTCAGTG AGCGTTGCAC CAGGTCAGAC
(51) CGCGCGTATC TCGTGTAGCG GCGATAATAT TGGTCATTAT TATGCTTCTT
(101) GGTACCAGCA GAAACCCGGG CAGGCGCCAG TTCTTGTGAT TTATCGTGAT
(151) AATGATCGTC CCTCAGGCAT CCCGGAACGC TTTAGCGGAT CCAACAGCGG
(201) CAACACCGCG ACCCTGACCA TTAGCGGCAC TCAGGCGGAA GACGAAGCGG
(251) ATTATTATTG CCAGTCTTAT GATTATCTTC ATGATTTTGT GTTTGGCGGC
(301) GGCACGAAGT TAACCGTTCT TGGCCAG
```

Figure 2b

Variable Light Chain Peptide

(CDR Regions in Bold)

3077_Vk kappa 2 (SEQ ID NO: 13):

(1) DIVMTQSPLS LPVTPGEPAS ISCRSSQ**SLL FIDGNNY**LNW YLQKPGQSPQ
(51) **LLIYLGSNRA** SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV **YYCQQYSSKS**
(101) ATFGQG**TKVE** IKRT

3079_Vk kappa 1 (SEQ ID NO: 14):

(1) DIQMTQSPSS LSASVGDRVT ITCRASQDIS **AFLN**WYQ**QKP** GKAPK**LLIYK**
(51) **VSNLQSGVPS** RFSGSGSGTD FTLTIS**SLQP** EDFATY**YCQQ** **AYSGSITFGQ**
(101) GTKVEIKRT

3080_VI lambda 3 (SEQ ID NO: 15):

(1) DIELTQPPSV SVAPGQTARI SCSGDN**IGNK** YVSWYQ**QKPG** QAPV**VVIYGD**
(51) **NNRPSGIPER** FSGSNSGNTA TLTISGTQAE DEADY**YC**SSY **DSSYFVF**GGG
(101) TKLTVLGQ

3100_VI lambda 3 (SEQ ID NO: 16):

(1) DIELTQPPSV SVAPGQTARI SCSGDN**IGHY** YASWYQ**QKPG** QAPV**LVIYRD**
(51) **NDRPSGIPER** FSGSNSGNTA TLTISGTQAE DEADY**YC**QSY **DYLHDFV**FGG
(101) GTKLTVLGQ

Figure 3

Variable Heavy Chain Consensus Sequences

(CDR Regions in Bold)

VH1B Consensus (SEQ ID NO: 17):

(1) QVQLVQSGAE VKKPGASVKV SCKASGYTFT **SYMHWVRQA** PGQGLEWMGW
(51) **INPNSGGTNY** **AQKFQGRVTM** TRDTSISTAY MELSSLRSED TAVYYCARWG
(101) **GDGFYAMDYW** **GQGLVTVSS**

VH3 Consensus (SEQ ID NO: 18):

(1) QVQLVESGGG LVQPGGSLRL SCAASGFTFS **SYAMSWVRQA** PGKGLEWVSA
(51) **ISGSGGSTYY** **ADSVKGRFTI** SRDNSKNTLY LQMNSLRAED TAVYYCARWG
(101) **GDGFYAMDYW** **GQGLVTVS** S

Figure 4

Variable Light Chain Consensus Sequences

(CDR Regions in **Bold**)

VL_λ3 Consensus (SEQ ID NO: 19):

(1) SYELTQPPSV SVAPGQTARI **SCSGDALGDK** **YASWYQQKPG** QAPVLVIYDD
(51) **SDRPSGIPER** **FSGSNSGNTA** **TLTISGTQAE** **DEADYYCQQH** **YTTPPVFGGG**
(101) TKLTVLG

VL_k1 Consensus (SEQ ID NO: 20):

(1) DIQMTQSPSS LSASVGDRVT ITCRASQGIS SYLAWYQQKP GKAPKLLIYA
(51) ASSLQSGVPS RFGSGSGTD FTLTISSLQP EDFATYYCQQ HYTTPPTFGQ
(101) GTKVEIKR

VL_k2 Consensus (SEQ ID NO: 21):

(1) DIVMTQSPLS LPVTPGEPAS ISCRSSQSLL HSNQYNYLDW YLQKPGQSPQ
(51) LLIYLGSNRA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCQQHYTTP
(101) PTFGQGTKVE IKR

Figure 5

Peptide Sequence of CD38

(SEQ ID NO: 22):

```
1      mancefspvs gdkpccrlsr raqlclgvs lvlilvvvla vvvprwrqqw sgpgttkrfp
61     etvlarcvky teihpemrhv dcqsvwdafk gafiskhpcn iteedyqplm klgtqtvpcn
121    killwsrikd lahqftqvqr dmftledtll gyladdltwc gefntskiny qscpdwrkdc
181    snnpvsfvwk tvsrrfaeaa cdvvhvmlng srskifdkns tfgsvevhn l qpekvtlea
241    wvihggreds rdlcqdptik elesiiskrn iqfsckniyr pdkflqcvkn pedssctsei
```

Figure 6

Nucleotide Sequence of Chimeric OKT10

Heavy Chain (SEQ ID NO: 23):

caggtggaat tgggtggaatc tggaggatcc ctgaaactct cctgtgcagc ctcaggattc
gatttttagta gatcctggat gaattgggtc cggcaggctc caggaaaagg gctagaatgg
attggagaaa ttaatccaga tagcagtacg ataaactata cgacatctct aaaggataaa
ttcatcatct ccagagacaa cgccaaaaat acgctgtacc tgcaaatgac caaagtgaga
tctgaggaca cagcccttta ttactgtgca agatatggta actggtttcc ttattggggc
caagggactc tggtcactgt cagctcagcc tccaccaagg gtccatcggc cttccccctg
gcacctcct ccaagagcac ctctgggggc acagcggccc tgggctgcct ggtcaaggac
tacttccccg aaccggtgac ggtgtcgtgg aactcaggcg ccctgaccag cggcgtgcac
accttccccg ctgtcctaca gtcctcagga ctctactccc tcagcagcgt ggtgaccgtg
ccctccagca gcttgggcac ccagacctac atctgcaacg tgaatcacia gccagcaac
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tgcccagcac ctgaactcct ggggggaccg tcagtcttcc tcttcccccc aaaacccaag
gacaccctca tgatctcccg gacccttgag gtcacatgcg tgggtggtgga cgtgagccac
gaagaccctg aggtcaagtt caactggtac gtggacggcg tggaggtgca taatgccaa
acaaagccgc gggaggagca gtacaacagc acgtaccggg tggtcagcgt cctcacgcgc
ctgcaccagg actggctgaa tggcaaggag tacaagtgca aggtctccaa caaagccctc
ccagccccc cagagaaaac catctccaaa gccaaagggc agccccgaga accacagggtg
tacaccctgc ccccatcccg ggatgagctg accaagaacc aggtcagcct gacctgcctg
gtcaaaggct tctatcccag cgacatcgcc gtggagtggg agagcaatgg gcagccggag
aacaactaca agaccacgcc tcccggtgtg gactccgacg gctccttctt cctctacagc
aagctcaccg tggacaagag caggtggcag caggggaacg tcttctcatg ctccgtgatg
catgaggctc tgcacaacca ctacacgcag aagagcctct ccctgtctcc gggtaaa

Light Chain (SEQ ID NO: 24):

gatatcctga tgaccagtc tcaaaaaatc atgccacat cagtgggaga cagggtcagc
gtcacctgca aggccagtc aaatgtggat actaatgtag cctggatatca acagaaacca

ggacagtctc ctaaagcact gatttactcg gcacccctacc gatacagtgg agtccctgat
cgcttcacag gcagtggatc tgggacagat ttcactctca ccatcaccaa tgtgcagtct
gaggacttgg cagagtattt ctgtcagcaa tatgacagct atcctctcac gttegggtgct
gggaccaagc tggacctgaa acgtacgggtg gctgcaccat ctgtcttcat cttcccggcca
tctgatgagc agttgaaatc tggaaactgcc tctgttgtgt gcctgctgaa taacttctat
cccagagagg ccaaagtaca gtggaagggtg gataacgccc tccaatcggg taactcccag
gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg
ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc
ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gt

Fig.7: Schematic Overview of Epitopes

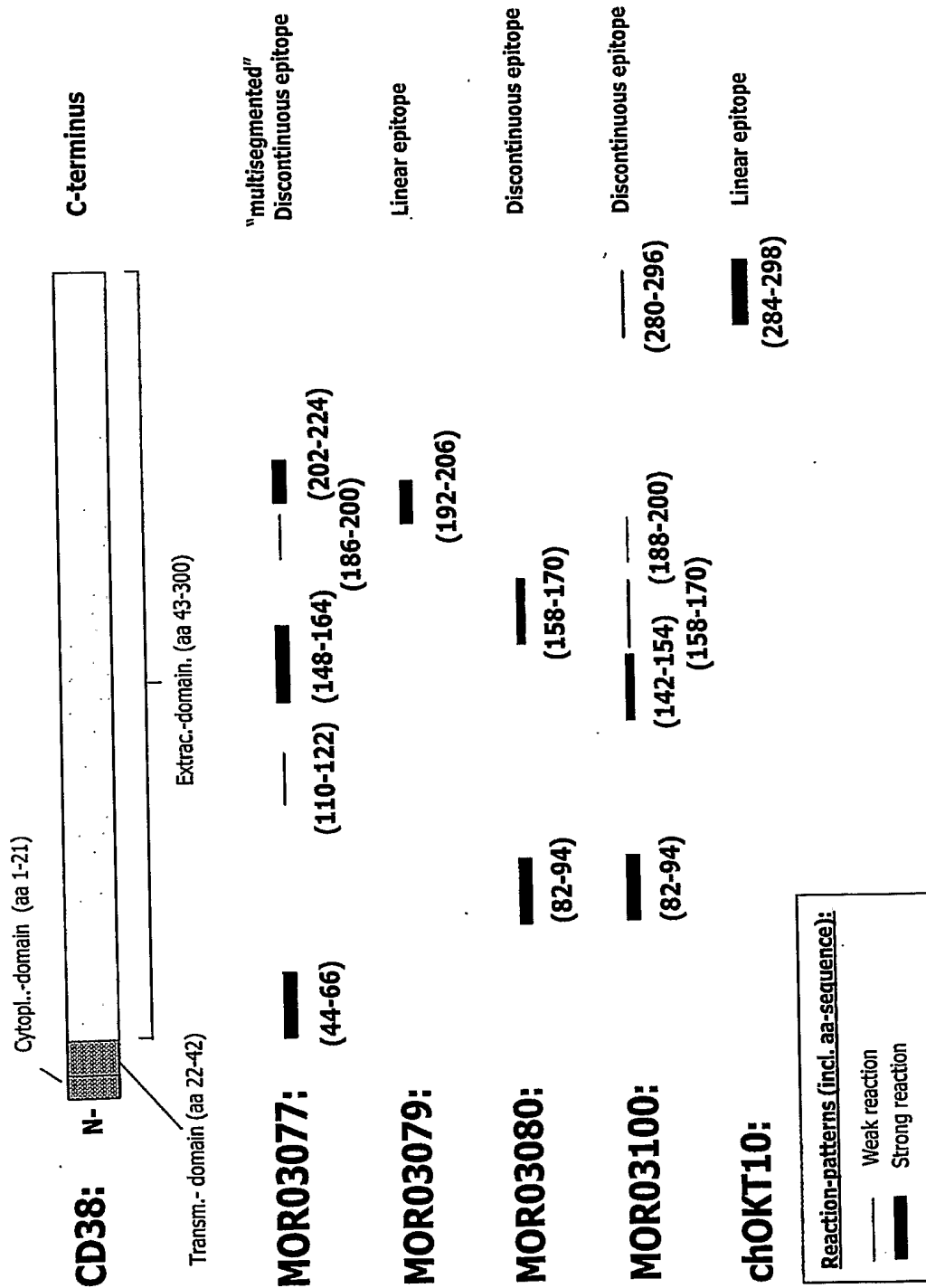


Figure 8: DNA sequence of pMOPRH®_h_IgG1_1

```

                StyI
                ~~~~~
601   TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA
      AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

                                   AatII
                                   ~~~~~
651   TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
      ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

701   TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
      ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

751   ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
      TGGTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

801   GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA CTGCTTACTG
      CAGATATATT CGTCTCGAGA GACCGATTGA TCTCTTGGGT GACGAATGAC

                pMORPH®_Ig_FOR 100.0%
                ~~~~~
851   GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGC
      CGAATAGCTT TAATTATGCT GAGTGATATC CCTCTGGGTT CGACCGATCG

      M K H L W F F L L L V A A P R .
901   GCCACCATGA AACACCTGTG GTTCTTCCTC CTGCTGGTGG CAGCTCCCAG
      CGGTGGTACT TTGTGGACAC CAAGAAGGAG GACGACCACC GTCGAGGGTC

                EcoRI                BlnI                StyI
                ~~~~~                ~~~~~                ~~~~~
      . W V L S Q V E F C R R L A Q A S T .
951   ATGGGTCTTG TCCCAGGTGG AATTCTGCAG GCGGTTAGCT CAGCCTCCAC
      TACCCAGGAC AGGGTCCACC TTAAGACGTC CGCCAATCGA GTCGGAGGTG

      StyI                BbsI
      ~~~~~                ~~~~~
      . K G P S V F P L A P S S K S T S G .
1001  CAAGGGTCCA TCGGTCTTCC CCCTGGCACC CTCCTCCAAG AGCACCTCTG
      GTTCCCAGGT AGCCAGAAGG GGGACCGTGG GAGGAGGTTT TCGTGGAGAC

      . G T A A L G C L V K D Y F P E P
1051  GGGGCACAGC GGCCCTGGGC TGCCTGGTCA AGGACTACTT CCCCGAACCG
      CCCCCTGTCTG CCGGGACCCG ACGGACCAGT TCCTGATGAA GGGGCTTGGC

```

V T V S W N S G A L T S G V H T F .
1101 GTGACGGTGT CGTGGAACTC AGGCGCCCTG ACCAGCGGCG TGCACACCTT
CACTGCCACA GCACCTTGAG TCCGCGGGAC TGGTCGCCGC ACGTGTGGAA

. P A V L Q S S G L Y S L S S V V T .
1151 CCCGGCTGTC CTACAGTCCT CAGGACTCTA CTCCCTCAGC AGCGTGGTGA
GGGCCGACAG GATGTCAGGA GTCTTGAGAT GAGGGAGTCG TCGCACCCT

. V P S S S L G T Q T Y I C N V N
1201 CCGTGGCCTC CAGCAGCTTG GGCACCCAGA CCTACATCTG CAACGTGAAT
GGCAGGGGAG GTCGTGCAAC CCGTGGGTCT GGATGTAGAC GTTGCACTTA

StyI
~~~~~  
H K P S N T K V D K K V E P K S C .  
1251 CACAAGCCCA GCAACACCAA GGTGGACAAG AAAGTTGAGC CCAAATCTTG  
GTGTTCCGGT CGTTGTGGTT CCACCTGTTT TTTCAACTCG GGTTTAGAAC

. D K T H T C P P C P A P E L L G G .  
1301 TGACAAAAC CACACATGCC CACCGTGCCC AGCACCTGAA CTCCTGGGGG  
ACTGTTTTGA GTGTGTACGG GTGGCAGGGG TCGTGGACTT GAGGACCCCC

BbsI StyI  
~~~~~  
. P S V F L F P P K P K D T L M I
1351 GACCGTCAGT CTTCTCTCTC CCCCCAAAAC CCAAGGACAC CCTCATGATC
CTGGCAGTCA GAAGGAGAAAG GGGGGTTTTG GGTTCCTGTG GGAGTACTAG

BbsI
~~~~~  
S R T P E V T C V V V D V S H E D .  
1401 TCCCGGACCC CTGAGGTCAC ATGCGTGGTG GTGGACGTGA GCCACGAAGA  
AGGGCCTGGG GACTCCAGTG TACGCACCAC CACCTGCACT CCGTGTCTCT

BbsI  
~  
. P E V K F N W Y V D G V E V H N A .  
1451 CCCTGAGGTC AAGTTCAACT GGTACGTGGA CGGCGTGGAG GTGCATAATG  
GGGACTCCAG TTCAAGTTGA CCATGCACCT GCCGCACCTC CACGTATTAC

. K T K P R E E Q Y N S T Y R V V  
1501 CCAAGACAAA GCCGCGGGAG GAGCAGTACA ACAGCACGTA CCGGGTGGTC  
GGTTCGTGTTT CGGCGCCCTC CTCGTGATGT TGTCGTGCAT GGCCCACCAG

S V L T V L H Q D W L N G K E Y K .  
1551 AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA AGGAGTACAA  
TCGCAGGAGT GGCAGGACGT GGTCTGACC GACTTACCGT TCCTCATGTT

. C K V S N K A L P A P I E K T I S .  
1601 GTGCAAGGTC TCCAACAAAG CCCTCCCAGC CCCCATCGAG AAAACCATCT  
CACGTTCCAG AGGTTGTTTC GGGAGGGTCG GGGGTAGCTC TTTTGGTAGA

BsrGI  
~~~~~  
. K A K G Q P R E P Q V Y T L P P
1651 CCAAAGCCAA AGGGCAGCCC CGAGAACCAC AGGTGTACAC CCTGCCCCCA
GGTTTCGGTT TCCCGTCGGG GCTCTTGGTG TCCACATGTG GGACGGGGGT

S R D E L T K N Q V S L T C L V K .
 1701 TCCCGGGATG AGCTGACCAA GAACCAGGTC AGCCTGACCT GCCTGGTCAA
 AGGGCCCTAC TCGACTGGTT CTGGTCCAG TCGGACTGGA CGGACCAGTT

. G F Y P S D I A V E W E S N G Q P .
 1751 AGGCTTCTAT CCCAGCGACA TCGCCGTGGA GTGGGAGAGC AATGGGCAGC
 TCCGAAGATA GGGTCGCTGT AGCGGCACCT CACCCTCTCG TTACCCGTCG

. E N N Y K T T P P V L D S D G S
 1801 CGGAGAACAA CTACAAGACC ACGCCTCCCG TGCTGGACTC CGACGGCTCC
 GCCTCTTGTT GATGTTCTGG TCGGAGGGC ACGACCTGAG GCTGCCGAGG

F F L Y S K L T V D K S R W Q Q G .
 1851 TTCTTCTCT ACAGCAAGCT CACCGTGGAC AAGAGCAGGT GGCAGCAGGG
 AAGAAGGAGA TGTCGTTTCA GTGGCACCTG TTCTCGTCCA CCGTCGTCCC

BbsI NsiI
 ~~~~~  
 . N V F S C S V M H E A L H N H Y T .  
 1901 GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCCTACA  
 CTTGCAGAAG AGTACGAGGC ACTACGTACT CCGAGACGTG TTGGTGATGT

SapI PmeI  
 ~~~~~  
 . Q K S L S L S P G K *
 1951 CGCAGAAGAG CCTCTCCCTG TCTCCGGGTA AATGAGGGCC CGTTTAAACC
 GCGTCTTCTC GGAGAGGGAC AGAGGCCCAT TTAATCCCGG GCAAATTG

2001 CGCTGATCAG CCTCGACTGT GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG
 GCGACTAGTC GGAGCTGACA CGGAAGATCA ACGGTCGGTA GACAACAAAC

~~~~~  
 pMORPH<sup>+</sup> Ig REV 100.0%  
 2051 CCCCTCCCCC GTGCCTTCCT TGACCCTGGA AGGTGCCACT CCCACTGTCC  
 GGGGAGGGGG CACGGAAGGA ACTGGGACCT TCCACGGTGA GGGTGACAGG

Figure 9: DNA Sequence of Ig kappy light chain expression vector pMORPH®\_h\_Igk\_1

```

                StyI
                ~~~~~
601 TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA
 AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

651 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
 ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

701 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
 ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

751 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
 TGTTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

801 GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA CTGCTTACTG
 CAGATATATT CGTCTCGAGA GACCGATTGA TCTCTTGGGT GACGAATGAC

 pMORPH®_Ig_FOR 100%
 =====
851 GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGC
 CGAATAGCTT TAATTATGCT GAGTGATATC CCTCTGGGTT CGACCGATCG

+1 M V L Q T Q V F I S L L L W I
 StyI
      ~~~~~
901  GCCACCATGG TGTTCAGAC CCAGGTCTTC ATTTCTCTGT TGCTCTGGAT
    CGGTGGTACC ACAACGTCTG GGTCCAGAAG TAAAGAGACA ACGAGACCTA

                BbsI
                ~~~~~

+1 S G A Y G D I V M I K R T V A A
 EcoRV
                ~~~~~
                BsiWI
                ~~~~~
951 CTCTGGTGCC TACGGGGATA TCGTGATGAT TAAACGTACG GTGGCTGCAC
 GAGACCACGG ATGCCCCTAT AGCACTACTA ATTTGCATGC CACCGACGTG

+1 P S V F I F P P S D E Q L K S G T
1001 CATCTGTCTT CATCTTCCCG CCATCTGATG AGCAGTTGAA ATCTGGAAC
 GTAGACAGAA GTAGAAGGGC GGTAGACTAC TCGTCAACTT TAGACCTTGA

 BbsI
                ~~~~~

```

```

+1 A S V V C L L N N F Y P R E A K V
1051 GCCTCTGTTG TGTGCCTGCT GAATAACTTC TATCCCAGAG AGGCCAAAGT
    CGGAGACAAC ACACGGACGA CTTATTGAAG ATAGGGTCTC TCCGGTTTCA

+1 Q W K V D N A L Q S G N S Q E S
1101 ACAGTGAAG GTGGATAACG CCCTCCAATC GGGTAACTCC CAGGAGAGTG
    TGTCACCTTC CACCTATTGC GGGAGGTTAG CCCATTGAGG GTCCTCTCAC

+1 V T E Q D S K D S T Y S L S S T L
1151 TCACAGAGCA GGACAGCAAG GACAGCACCT ACAGCCTCAG CAGCACCCTG
    AGTGTCTCGT CCTGTCTGTC CTGTCTGTGA TGTCGGAGTC GTCGTGGGAC

+1 T L S K A D Y E K H K V Y A C E V
    BlnI
    ~~~~~
1201 ACGCTGAGCA AAGCAGACTA CGAGAAACAC AAAGTCTACG CCTGCGAAGT
 TGCGACTCGT TTCGTCTGAT GCTCTTTGTG TTTCAGATGC GGACGCTTCA

+1 T H Q G L S S P V T K S F N R G
1251 CACCCATCAG GGCCTGAGCT CGCCCGTCAC AAAGAGCTTC AACAGGGGAG
 GTGGGTAGTC CCGGACTCGA GCGGGCAGTG TTTCTCGAAG TTGTCCCCTC

+1 E C *
 PmeI
    ~~~~~
    pMORPH®_Ig_REV 100%
    =====
1301 AGTGTTAGGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT
    TCACAATCCC CGGGCAAATT TGGGCGACTA GTCGGAGCTG ACACGGAAGA

=
1351 AGTTGCCAGC CATCTGTTGT TTGCCCCTCC CCCGTGCCTT CTTGACCCT
    TCAACGGTCG GTAGACAACA AACGGGGAGG GGGCACGGAA GGAACGGGA

```

**Figure 10: DNA Sequence of HuCAL® Ig lambda light chain vector pMORPH®\_h\_Igλ\_1**

```

                StyI
                ~~~~~
601 TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA
 AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

651 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
 ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

701 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
 ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

751 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
 TGTTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

801 GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA CTGCTTACTG
 CAGATATATT CGTCTCGAGA GACCGATTGA TCTCTGGGT GACGAATGAC

 pM_Ig_FOR 100.0%
                ~~~~~
851  GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGC
    CGAATAGCTT TAATTATGCT GAGTGATATC CCTCTGGGT CGACCGATCG

+1      M A W A L L L L T L L T Q G T
        StyI
        ~~~~~
901 GCCACCATGG CCTGGGCTCT GCTGCTCCTC ACCCTCCTCA CTCAGGGCAC
 CGGTGGTACC GGACCCGAGA CGACGAGGAG TGGGAGGAGT GAGTCCCGTG

+2 T V L G Q
+1 G S W A D I V M H E V
 BamHI EcoRV HpaI StyI
        ~~~~~
951  AGGATCCTGG GCTGATATCG TGATGCACGA AGTTAACCGT CCTAGGTCAG
    TCCTAGGACC CGACTATAGC ACTACGTGCT TCAATTGGCA GGATCCAGTC

+2      P K A A P S V T L F P P S S E E L
        StyI
        ~~~~~
1001 CCCAAGGCTG CCCCTCGGT CACTCTGTTC CCGCCCTCCT CTGAGGAGCT
 GGGTTCCGAC GGGGGAGCCA GTGAGACAAG GGCGGGAGGA GACTCCTCGA

+2 Q A N K A T L V C L I S D F Y P
1051 TCAAGCCAAC AAGGCCACAC TGGTGTGTCT CATAAGTGAC TTCTACCCGG
 AGTTCGGTTG TTCCGGTGTG ACCACACAGA GTATTCACTG AAGATGGGCC

```

+2 G A V T V A W K G D S S P V K A G  
 1101 GAGCCGTGAC AGTGGCCTGG AAGGGAGATA GCAGCCCCGT CAAGGCGGGA  
 CTCGGCACTG TCACCGGACC TTCCCTCTAT CGTCGGGGCA GTTCCGCCCT

+2 V E T T T P S K Q S N N K Y A A S  
 1151 GTGGAGACCA CCACACCCTC CAAACAAAGC AACAAACAAGT ACGCGGCCAG  
 CACCTCTGGT GGTGTGGGAG GTTTGTTTCG TTGTTGTTCA TGCGCCGGTC

+2 S Y L S L T P E Q W K S H R S Y  
 1201 CAGCTATCTG AGCCTGACGC CTGAGCAGTG GAAGTCCCAC AGAAGCTACA  
 GTCGATAGAC TCGGACTGCG GACTCGTCAC CTTCAGGGTG TCTTCGATGT

+2 S C Q V T H E G S T V E K T V A P  
 BbsI

1251 GCTGCCAGGT CACGCATGAA GGGAGCACCG TGGAGAAGAC AGTGGCCCCCT  
 CGACGGTCCA GTGCGTACTT CCCTCGTGGC ACCTCTTCTG TCACCGGGGA

+2 T E C S \*

PmeI

1301 ACAGAATGTT CATAGGGGCC CGTTTAAACC CGCTGATCAG CCTCGACTGT  
 TGTCTTACAA GTATCCCCGG GCAAATTTGG GCGACTAGTC GGAGCTGACA  
 pM\_Ig\_REV 100%  
 =====

1351 GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC GTGCCTTCCT  
 CGGAAGATCA ACGGTCGGTA GACAACAAAC GGGGAGGGGG CACCGAAGGA  
 pM\_Ig\_REV 100.0%  
 =====

## **Application Data Sheet**

### **Application Information**

|                                  |                                              |
|----------------------------------|----------------------------------------------|
| Application number::             | Unassigned                                   |
| Filing Date::                    | February 26, 2004                            |
| Application Type::               | Utility                                      |
| Subject Matter::                 |                                              |
| Suggested classification::       |                                              |
| Suggested Group Art Unit::       |                                              |
| CD-ROM or CD-R?::                | No                                           |
| Number of CD disks::             |                                              |
| Number of copies of CDs::        |                                              |
| Sequence submission?::           | Yes                                          |
| Computer Readable Form (CRF)?::  | No                                           |
| Number of copies of CRF::        |                                              |
| Title::                          | Anti-CD38 Human Antibodies and Uses Therefor |
| Attorney Docket Number::         | 37629-0085A                                  |
| Request for Early Publication?:: | No                                           |
| Request for Non-Publication?::   | No                                           |
| Suggested Drawing Figure::       |                                              |
| Total Drawing Sheets::           |                                              |
| Small Entity?::                  | Yes                                          |
| Latin name::                     |                                              |
| Variety denomination name::      |                                              |
| Petition included?::             | No                                           |
| Petition Type::                  |                                              |
| Licensed US Govt. Agency::       |                                              |
| Contractor Grant Numbers::       |                                              |
| Secrecy Order in Parent Appl.?:: | No                                           |

## Applicant Information

|                                         |          |
|-----------------------------------------|----------|
| Applicant Authority Type::              | Inventor |
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| Status::                                |          |
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| Middle Name::                           |          |
| Family Name::                           | Tesar    |
| Name Suffix::                           | Dr.      |
| City of Residence::                     | Weilheim |
| State or Province of Residence::        |          |
| Country of Residence::                  | Germany  |
| Street of mailing address::             |          |
| City of mailing address::               | Weilheim |
| State or Province of mailing address::  |          |
| Country of mailing address::            | Germany  |
| Postal or Zip Code of mailing address:: |          |

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|                                  |       |  |
|----------------------------------|-------|--|
| Representative Customer Number:: | 26633 |  |
|----------------------------------|-------|--|

- OR -

| Representative Designation:: | Registration Number:: | Representative Name:: |
|------------------------------|-----------------------|-----------------------|
|                              |                       |                       |
|                              |                       |                       |
|                              |                       |                       |
|                              |                       |                       |